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EFFECTS OF GRAZING INTENSIFICATION AND ABANDONMENT ON THE COMPOSITION, DIVERSITY AND FUNCTIONING OF MEDITERRANEAN GRASSLANDS.

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A mis padres.

A Carol.

Presentación

Los cambios en el uso del suelo son uno de los principales causantes del proceso de cambio global que amenaza la provisión de servicios ecosistémicos para los seres humanos. Entre los distintos usos del suelo, los sistemas ganaderos, probablemente el uso más extendido y el que demanda más recursos a nivel global, han experimentado grandes cambios en las últimas décadas; cambios que se espera que continúen en un futuro cercano debido al aumento de la demanda mundial. El proceso simultáneo de intensificación y abandono que afecta a los pastos europeos – y muy especialmente a los mediterráneos – compromete la diversidad y la capacidad de proveer servicios de estos ecosistemas. Históricamente, el pastoreo ha estado muy estrechamente ligado a los ecosistemas mediterráneos, que se han visto afectados por la actividad de los herbívoros domésticos durante milenios. Debido a esto, el pastoreo ha sido reconocido como un factor esencial para la conservación de estos sistemas, por lo que es de especial interés conocer cuáles son las consecuencias de los procesos de intensificación y abandono.

Puesto que el pastoreo afecta a la vegetación a través de diversos mecanismos, y dado que sus efectos se manifiestan a distintos niveles, que van desde las plantas individuales hasta el nivel de paisaje, el estudio de las consecuencias de los cambios de uso en sistemas ganaderos requiere el examen simultáneo de diferentes aspectos. La presente tesis doctoral tiene como principal objetivo describir los efectos que el proceso dual de intensificación y abandono ganadero tiene en la composición, funcionamiento y persistencia de los pastos mediterráneos, centrándose específicamente en el estudio de las dehesas, dado que son de uno de los ejemplos más representativos de estos ecosistemas.

Los seis manuscritos que componen esta tesis doctoral analizan estos efectos en un orden de detalle decreciente, comenzando con los efectos que el pastoreo tiene en las especies. De este modo, en el CAPÍTULO 2 se analizan los efectos que los lixiviados tienen en la germinación de semillas y desarrollo inicial de plántulas de especies con distinta respuesta al pastoreo. Los siguientes tres capítulos analizan los efectos de la intensificación y el abandono en las comunidades de pastizales. En el CAPÍTULO

3, se estudian las consecuencias que el abandono tiene en la diversidad de especies, la diversidad funcional, la composición de especies y algunas características ambientales. Posteriormente se analiza el papel que tiene la variabilidad espacial y temporal en la disponibilidad de recursos como moduladora de la respuesta al pastoreo de las comunidades vegetales. El CAPÍTULO 4 se dedica al estudio de la variabilidad interanual en la disponibilidad hídrica y su interacción con la intensidad de pastoreo y el tipo de hábitat para determinar los procesos de ensamblaje de las comunidades vegetales. El CAPÍTULO 5 se centra en los efectos del pastoreo y la disponibilidad de recursos en la diversidad taxonómica y funcional a diferentes escalas espaciales. En el CAPÍTULO 6 se presenta una metodología para la gestión de sistemas ambientalmente heterogéneos como los pastizales mediterráneos. Posteriormente, en el CAPÍTULO 7, se estudian los efectos del abandono de las practicas trashumantes en la regeneración y el estado vegetativo del arbolado en dehesas de encina. Finalmente, en el CAPÍTULO 8, se recopilan y discuten los principales resultados y se proponen algunas líneas para futuras investigaciones.

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Chapter 1

Introduction

1.1 GRAZING INTENSIFICATION IN THE CONTEXT OF GLOBAL CHANGE

Humans dominate Earth. Unprecedented growth rates have taken the world population to nearly 7 billion people, an amount that is predicted to keep growing during the next century (United Nations Population Division 2010; Bloom 2011). The expansion experimented in the XX century has entailed increased volumes of food, water, housing and energy needed to satisfy the needs of humanity (Steffen *et al.* 2011). But the extraordinary growth of the utilization of resources has severely impacted ecosystems throughout the world, which are undergoing rapid environmental changes (MEA 2005). As a consequence, world's ecosystems are experiencing dramatic losses in biodiversity driven by habitat change, climate change, invasive alien species, overexploitation, and pollution (MEA 2005). Eventually, changes in biodiversity are likely to influence ecosystem processes and reduce the resilience of ecosystems to environmental change and their capacity to provide services for humans (Chapin *et al.* 2000). To improve the understanding on how the different drivers of global change affect ecosystems has become one of the biggest challenges for scientists and policy makers, and one that should be faced urgently (Sala *et al.* 2000; Tylianakis *et al.* 2008; Barnosky *et al.* 2012).

During the last three centuries, the prominence of managed grazing has experienced a remarkable increase and at the present time it is the most widespread land use at a global scale, occupying a 25% of the global surface (Asner *et al.* 2004). Globally, meat and milk produc-

tion has increased a 40% in the last 30 years and this trend is likely be maintained in the next decades, due to the increasing human population and the dietary shifts towards higher proportions of meat (Tilman *et al.* 2002; Bouwman *et al.* 2005; Robinson *et al.* 2011). However, the proportion of grassland surfaces have not increased in parallel to the production, a tendency that is not likely to change in the next years (Bouwman *et al.* 2005). This denotes that increased levels of agricultural intensification are needed in order to achieve the required production levels. Intensification processes involve the use of external inputs in livestock systems, such as fertilizers or externally produced feedstuffs. Thus, when the large quantity of croplands devoted to animal feed is taken into account along with pasture and grazing lands, the percentage of the world's agricultural land that is currently used for animal production adds up to 75% (Foley *et al.* 2011). In Europe, the tendency towards more intensified grazing practices has resulted in a dual process of abandonment in the less productive areas and intensification in the more productive or accessible ones (McDonald *et al.* 2000; Schmitzberger *et al.* 2005; Stoate *et al.* 2009). The complex changes experienced in the last decades are threatening the diversity and ecosystem services provided farmlands and grasslands across Europe (Stoate *et al.* 2009) and especially in Mediterranean environments (Sluiter and Jong 2006).

Grazing in the Mediterranean Basin: the dehesa system

Multifunctional agrarian social-ecological systems, where the interaction between human populations and nature has shaped landscapes for millennia (Papanastis 1998; Blondel and Aronson 1999; Di Pasquale *et al.* 2005), are largely responsible for the high biodiversity of the Mediterranean Basin (di Castri 1981; Perevolotsky and Seligman 1998). Among these landscapes, dehesas, Mediterranean open woodlands resulting from the clearing of original evergreen oak woodland and shrubland areas (Joffre *et al.* 1999; Plieninger 2007), also known as montados in Portugal, constitute an example of agrosilvopastoral ecosystems with a high biodiversity and have been widely presented as the paradigm of a sustainable land-use (e.g. Gómez-Limón and de Lucío Fernández 1999; Valladares 2007; Pinto-Correia *et al.* 2011). Dehesas are characterised by a two-layered structure composed of trees (generally evergreen oaks) over herbaceous vegetation (either semi-natural grasslands or crops; Joffre *et al.* 1999). These systems occupy a large surface in the Iberian Peninsula

and its conservation is considered very important for both environmental and socio-economic reasons (Moreno and Pulido 2009). Traditionally, dehesas have been grazed by domestic livestock, mainly sheep and cattle, at low stocking rates (Peco *et al.* 2006b). Livestock feed on the acorns and grass under the canopy of semiopen woodland, which also provides firewood, hence the trees are viewed as an integrated part of the system, and as a result are managed and regularly pruned (Montoya 1980; Joffre *et al.* 1999; Blondel 2006).

Mediterranean climate is characterized by marked differences between wet winters and dry summers with great inter-annual variability in rainfall. These climatic conditions have clear effects in the composition and productivity of Mediterranean grasslands. Annual species are very important in the less favourable areas in these systems (Naveh and Whittaker 1979; Ortega *et al.* 1997), because of their capacity to grow rapidly under changing environmental conditions (Grime 1977). However, in mountains or areas with a short or less intense summer drought, the relative importance of annuals decreases in favour of perennial species (Ortega *et al.* 1997).

The exploitation of dehesas has been traditionally associated to trashumance, which consist in a regular and periodic movement of livestock from winter pastures (valley bottoms and/or southern latitudes) to summer pastures (mountains and/or northern latitudes; Weber and Horst 2011). The climatic conditions, along with the high topographic variability, make transhumance a successful grazing management practice in these environments (Niamir-Fuller and Turner 1999; Manzano and Casas 2010; Weber and Horst 2011). Transhumant practices optimize the exploitation of the existing resources, by being present in each region during the period of time in which its production maximizes (Ruiz and Ruiz 1986). Because of its size, climate, topography and anthropological characteristics, transhumance systems have historically had a great importance in the Iberian Peninsula (Manzano and Casas 2010; Ruiz and Ruiz 1986).

Intensification in dehesas

As part of the generalized intensification process, extensive grazing practices are being substituted by intensive regimes in the Mediterranean Europe. This substitution is characterized by the preference for large-scale free range grazing over traditional shepherding and the gradual loss of many traditional cultural practices (Pinto-Correia 1999; Vos

and Meekes 1999; Peco *et al.* 2001). Despite its adaptive nature and wide distribution in the past, transhumance has been disappearing in the Mediterranean, especially over the last 50 years. Dehesas, that have traditionally been the wintering areas for transhumant herds, are currently facing deterioration because of the dual intensification/abandonment process (Peco *et al.* 2001). Intensification and abandonment can coexist in a given area, and even on a single farm, which makes it more difficult to monitor their effects on the dehesas environmental quality (Peco *et al.* 1999).

Many dehesas are threatened because of the overexploitation of estates, which is fairly related with the sedentarization of previously transhumant herds (Le Houerou 1977) and the increased stocking levels based in grain and fodder supply (Peco *et al.* 2001; Plieninger *et al.* 2004). Similarly to other landscapes composed by scattered trees, the last decades have seen a remarkable decrease in the total area covered by dehesas as well as in the tree densities of the remaining ones (Moreno and Pulido 2009). Studies on the size structure of dehesas have shown that these systems are characterized by over-aged oak stands, because of a disruption in the forest cycle characterized by an extensive lack of regeneration (Montoya 1998; Plieninger *et al.* 2003; Plieninger 2007). Whether the lack of regeneration of trees in dehesas is caused by recent intensification processes or is an inherent feature of grazed dehesas independent of grazing intensity is a question that has not been definitely answered to date (Moreno and Pulido 2009; Plieninger *et al.* 2011). However, regardless of its causes, lack of regeneration of the tree layer is threatening the long-term stability of dehesas, because of the capital importance that oaks have on these systems (Joffre *et al.* 1999; Plieninger *et al.* 2003).

At the same time, the increasing levels of grazing abandonment experienced by many Mediterranean areas are resulting in an extensive substitution of palatable herbaceous vegetation by woody vegetation (García and Lasanta 1992; Peco *et al.* 1993; Beaufoy *et al.* 1994; Bartolomé *et al.* 2005). This substitution bring several consequences, like a decrease in species diversity at the landscape scale (Noy-Meir *et al.* 1989; Bernáldez 1991; Peco *et al.* 2006b), reductions in soil fertility (Peco *et al.* 2006b) and water availability (Otero *et al.* 2011) and increased fire risk due to the accumulation of organic matter (Bernáldez 1991; Moreira *et al.* 2001,2007), associated with the subsequent risk of erosion (Peco *et al.* 2001). Besides these biophysical changes, the loss of traditional agricultural practices

is associated with the loss of cultural heritage and traditional ecological knowledge (Bunce *et al.* 2004; Oteros-Rozas *et al.* 2012)

1.2 GRAZING EFFECTS ON PLANTS

Extensive grazing by large herbivores has a decisive effect on the composition and structure of plant communities in Mediterranean grasslands (Noy-Meir *et al.* 1989; Peco *et al.* 2005; de Bello *et al.* 2007). Grazing plays a fundamental role in shaping and maintaining Mediterranean vegetation in different ways, such as consuming leaves, flowers, fruits and roots, trampling and also through dung and urine deposition.

These actions have different effects on the fitness and survivorship of plants. Losses of photosynthetic tissue due to defoliation frequently reduce the growth, reproductive performance and survival of the affected individuals (Karban and Strauss 1993; Del-Val and Crawley 2005; Staaldduinen *et al.* 2010). In order to cope with defoliation, plants adopt two main strategies: avoidance and tolerance (Anderson and Briske 1995). Avoidance strategies seek to minimise the '*frequency and/ or intensity of herbivory by reducing plant palatability and accessibility*' (Anderson and Briske 1995:1015), while tolerance to herbivory is defined as the capacity of plants to rapidly regrow after defoliation. Along with direct effects on plants, defoliation is also likely to influence species performance by modifications in the environmental conditions, such as changing the quantity and quality of light (Peco *et al.* 2006b; Dobarro *et al.* 2010). Trampling also removes plant tissues, leading to the formation of gaps that are afterwards colonised by relatively poor competitor species that take advantage of the reduced competition for light in gaps (Kohler *et al.* 2006). When grazing pressure is too high, trampling can lead to excessive losses in plant cover and increased levels of soil compaction, increasing the risk of soil erosion and productivity loss (Bernáldez and Peco 1991; Thornes 2007). Urine and dung deposition favours the formation of patches of some plant species (Dai 2000; Gillet *et al.* 2010) and fertilises the soils of the grazed areas (Peco *et al.* 2006b). Dung deposition is acknowledged as an important mechanism of seed dispersal (Malo and Suárez 1995b; Mouissie *et al.* 2005: Iravani *et al.* 2011), and one that is likely to affect species composition in grasslands because of the great interspecific differences in the ability to survive and germinate after ingestion by herbivores (Peco *et al.* 2006a; D'hondt and Hoffmann 2011). Finally, grazers

disperse seeds that get attached to their skins (Fischer *et al.* 1996; de Pablos and Peco 2007; Couvreur *et al.* 2008), a dispersal mechanism that can reach extreme long-distances (Manzano and Malo 2006).

These grazing activities affect plant colonization and extinction rates in plant communities (Olff and Ritchie 1998), acting as a filter for their species. The role played by herbivores in the control of plant diversity has been widely recognized as a fundamental issue in the management of pastoral systems (e.g. Peco *et al.* 2006b; de Bello *et al.* 2007; Golodets *et al.* 2011). For example, several grassland species, including some Mediterranean endemics, are limited to grazed or non-grazed sites. Such species are usually labelled as grazing increasers or decreasers, depending on their presence-absence or abundance response to grazing in a given zones (Vesk and Westoby 2001). Many studies have explained these responses on the basis of mechanisms that differ between species, including tolerance to herbivory (Anderson and Briske 1995; del-Val and Crawley 2004 2005; Bonanomi *et al.* 2006; Wise and Abrahamson 2007), herbivore selectivity (Anderson and Briske 1995; Cingolani *et al.* 2005b), herbivore seed dispersal (Malo and Suárez 1995b; Will and Tackenberg 2008), plant-microbe interaction (Dormaar *et al.* 2002) and herbivore disturbance of the nutrient cycle (Peco *et al.* 2006b; Holdo *et al.* 2007; Tárrega *et al.* 2009). Some studies suggest that increaser species have higher growth rates than decreasers (Leoni *et al.* 2009), a feature that results in a higher capacity to tolerate biomass removal by herbivores (del-Val and Crawley 2004,2005). Additionally, some studies have found differences in the germination response between increasers and decreasers species in relation to light availability (Dobarro *et al.* 2010)

As indicated above, Mediterranean grasslands are often dominated by annual plants (Peco *et al.* 2006b), a feature that underscores the role that germination and seedling establishment have on their specific composition (Espigares *et al.* 1995; Marañón 1998; Peco *et al.* 2009). Although it is acknowledged that dung deposition can have a major effect on seed dispersal (Malo and Suárez 1995b) and that there are great differences between species in their ability to survive and germinate after ingestion by herbivores (Peco *et al.* 2006a; D'hondt and Hoffmann 2011), the effect of dung leachates on seeds already present in the seed bank has not been tested before. Cattle dung is a major source of nutrients and phytotoxic compounds (Hoekstra *et al.* 2002), some of which are likely to affect plant germination and initial growth (Hillhorst and Karssen 2000; Farnsworth 2008; Berendse and Möller 2009; Luna and Moreno 2009).

Breaking seed dormancy often depends on environmental conditions, and thus different species have different germination patterns depending on their environmental preferences (Vandelook *et al.* 2008). The differential effect of dung on the germination and establishment of different groups of species may therefore be one of the mechanisms responsible for the different species compositions in grazed and ungrazed environments. To date, no experimental study has considered the effect of dung on germination as a potential explanation for different species' response to grazing.

1.3 DIVERSITY AND ECOSYSTEMS FUNCTIONING

In the last years, there is an increasing alarm caused by the observed reduction in the supply of ecosystem services that ultimately support human wellbeing (MEA 2005; Balvanera *et al.* 2006). In this global-change context, the development of models to predict the effects that land-use change will have on the provisioning of ecosystem services has become an urgent task for science (Schröter *et al.* 2005; Foley *et al.* 2005; Petchey and Gaston 2006). It is generally recognised that biological diversity, along with other factors, drives ecosystems functioning and processes, which ultimately determine the provision of ecosystem services (Hooper and Vitousek 1997; Tilman *et al.* 1997; Díaz and Cabido 2001; Naeem and Wright 2003; Maestre *et al.* 2012). However, several studies have described that biodiversity is being lost at accelerated rates in the last decades (Chapin *et al.* 2000; Loreau *et al.* 2001; Steffen *et al.* 2011), and among the different components of global change, land use change is considered the most important driver of biodiversity loss and ecosystem services changes throughout the world (Sala *et al.* 2000; MEA 2005). It is important to note that diversity includes different components (i.e. taxonomical, functional and genetic diversity; Hooper *et al.* 2001; Petchey and Gaston 2006; Meynard *et al.* 2011).

As indicated above, the effects of grazing differ between plant species. Besides, these effects are modulated by environmental conditions, which can determine its strength and even its direction (Wise and Abrahamson 2005,2007,2008). Moreover, grazing and environment can also influence the interactions among coexisting species (e.g. Smit *et al.* 2007). Finally, all these factors scale-up to determine the identity

and abundance of the species present in grassland communities, and are finally manifested as effects on the diversity of species, not only at the community level, but also at greater escales (de Bello *et al.* 2006; Golodets *et al.* 2011).

Taxonomical diversity

Among the components of diversity, taxonomical diversity, usually estimated by species richness, has been the aspect that has received more attention in the scientific literature. Several models have been developed in order to describe the effects that productivity and disturbance rate have on species richness (Grime 1979; Huston 1979,1994; Rosenzweig and Abramsky 1993; Tilman and Pacala 1993). Herbivores affect the colonization and extinction rates of grassland species, being its final effect on species richness modulated by factors such as productivity or herbivore body size (Milchunas *et al.* 1988; Proulx and Mazumder 1998; Olff and Ritchie 1998; Bakker and Olff 2003; de Bello *et al.* 2005; Cingolani *et al.* 2005a; Bakker *et al.* 2006). The main conclusion of these studies is that grazing promotes species diversity when productivity levels are high, by reducing the relative importance of competitively dominant species and increasing subordinate species, while it reduces species diversity in less productive conditions, by increasing the mortality of the most palatable species (Bakker and Olff 2003). Most of these studies have been developed based on global or regional observations and thus it is not clear whether local differences in productivity within a climatic region can affect the relationship between species diversity and grazing (but see Sasaki *et al.* 2009a). Moreover, the effect of grazing in a given community could also change over time, influenced by temporal changes in the availability of resources. Mediterranean grasslands are appropriate for studying these questions, first, because in these systems small changes in productivity can change the direction of the effect of grazing on diversity (Bakker *et al.* 2006) and, second, because of the high interannual variability in rainfall associated with Mediterranean climate.

Functional diversity

In the last years, several studies are revealing that functional diversity, i.e. the value and range of the functional traits[1] of the species present in

[1]Despite the extensive use of the term *trait* in the literature, there is much confusion regarding its definition, and the underlying concepts involved. According to Violle *et*

a community (Diaz and Cabido 2001), is the aspect of diversity that is more strongly linked with ecosystem processes, and thus the one with a more direct influence on the provision of ecosystem services (Tilman *et al.* 1997, Díaz and Cabido 2001; Naeem and Wright 2003; Chapin 2003; Hooper *et al.* 2005; Quétier *et al.* 2007). Moreover, the use of approaches based on functional diversity rather than on species identities, permits the comparison between different systems, necessary in order to predict the effects of the different drivers of global change on the functioning of ecosystems (Chapin 2003; Lavorel *et al.* 2007; Violle *et al.* 2007). As a result, recent years have seen an increasing interest on the study of functional diversity and on how it is affected by environmental conditions and management (de Bello *et al.* 2006; Sasaki 2009b; Mayfield 2010). Nevertheless, more studies that relate the response of functional diversity to changes in land use (Flynn *et al.* 2009; Laliberté *et al.* 2010) or climatic conditions (Thullier *et al.* 2006) are necessary in order to predict changes in ecosystem processes associated with these drivers of global change.

It remains unclear what are the effects that the changes in land use associated to intensification processes have on ecosystem functioning. In order to maintain and promote ecosystem functioning, conservation measures often focus on conserving high species diversity, assuming that taxonomical and functional diversities are positively correlated. Thus, it is generally believed that land use-changes associated to intensification processes lead to a loss of species that result in a similar loss of functional diversity. Despite this assumption is true in some situations (e.g. Bowker *et al.* 2008), it is not necessarily so in all ecosystems and circumstances, because the loss of species is not necessarily associated with reductions in functional diversity (Mayfield *et al.* 2010). As such, the relationship between the taxonomical and functional aspects of diversity provides insights into the consequences that land use change can have on community assembly and ecosystem functioning (Sasaki 2009b). Thus, the characterization of this relationship and of the factors that modulate it has become a fundamental topic in conservation ecology (Biswas and Mallick 2011).

al. (2007) a *trait* is '*any morphological, physiological or phenological feature measurable at the individual level, from the cell to the whole-organism level, without reference to the environment or any other level of organization*'. However, only the traits that impact '*fitness indirectly via its effects on growth, reproduction and survival*' can be considered *functional traits*.

1.4 THE ROLE OF FUNCTIONAL TRAITS IN COMMUNITY ASSEMBLY

One of the fundamental aims of ecology is to understand how local communities are assembled from the regional pool of species (Diamond 1975; Weiher and Keddy 1995; Gotelli and McCabe 1999; Cornwell and Ackerly 2009). Two apparently opposing forces act simultaneously to determine the outcome of the assembly process: abiotic external forces (environmental filtering) and biotic internal forces (limiting similarity; Weiher *et al.* 1998; Grime 2006; Cornwell and Ackerly 2009; de Bello *et al.* 2012). Environmental filtering refers to the constraint that environmental conditions impose to the viable trait values at a given site (Diaz *et al.* 1998; Weiher *et al.* 1998; Pakeman *et al.* 2011; Mason *et al.* 2012). Limiting similarity prevents coexisting species from being too similar (Grime 2006; Mason *et al.* 2012,in press). Despite these processes can be interpreted as mutually exclusive, there is growing evidence that they act hierarchically determining the traits values allowed in a community and therefore the identities of the species. First, environmental filtering restricts the range of functional traits allowed in the community, selecting for a subset of the regional pool of species. Then, limiting similarity promotes functional differentiation among those species (Weiher *et al.* 1998; Cornwell and Ackerly 2009; de Bello *et al.* 2012). Because environmental filtering is predicted to reduce functional diversity and limiting similarity is predicted to increase it (Weiher and Keddy 1995), the relative importance of these mechanisms in the assembly of ecological communities can be inspected by studying the relation between functional traits and the abundance of species (Mouillot *et al.* 2007; de Bello *et al.* 2012; Mason *et al.* in press).

According to Mayfield *et al.* (2010), changes in land uses, such as grazing intensification or abandonment, impact upon community assembly processes, not species per se. This means that species diversity should be considered an outcome of community assembly processes. In the last years, the use of approaches related with functional traits along with the development of statistical techniques to analyse the observed patterns have vastly improved our understanding of the mechanisms that control community assembly processes and maintain biodiversity, which is a major objective for community ecology (Gotelli and McCabe 2002; Grime 2006; Wilson 2007; Mason *et al.* 2012; Spasojevic *et al.* 2012). Among the factors that can affect assembly processes, tem-

poral and spatial variations in resource availability are among the most studied (e.g. Mason *et al.* 2012; Spasojevic *et al.* 2012). However, the effect that short-term variability in resource availability can have on the assembly of biotic communities is an issue that has not been studied to date, despite it can be one of the mechanisms that promotes long-term coexistence of species in habitats with high temporal variability in resource availability. Mediterranean grasslands are an adequate system to study this question, due to the high interannual variability in rainfall, which causes a high temporal variability in their species composition grasslands (Peco 1989; Espigares and Peco 1993,1995; Peco *et al.* 1998).

1.5 THE LANDSCAPE SCALE: REMOTE SENSING BASED MONITORING

Grazing intensification can also have a major impact on Mediterranean dehesas at the landscape scale. This is because, depending on its intensity and on habitat productivity, grazing can either enhance soil fertility and productivity or be an important agent of land degradation (Peco *et al.* 2006b; Thornes 2007; Thompson *et al.* 2009; Kleinebecker *et al.* 2011). Due to the generalization of sedentary systems, the increases in stocking rates and the abandonment of shepherding practices, the problem of overgrazing is becoming more important in semiarid environments, like Mediterranean ones, especially in the vicinity of areas in which animals tend to concentrate, such as sheds or watering points (Röder *et al.* 2007). The increased levels of trampling and defoliation associated with overgrazing reduce vegetation cover, increasing the risk of soil erosion and increase the compaction of soils, thus decreasing infiltration rates and increasing run-off (Pickup and Chewings 1994; Thornes 2007). Subsequently, negative feedbacks can further accelerate the levels of land degradation in these habitats (Rietkerk *et al.* 2000; Kéfi *et al.* 2007).

Rangeland monitoring is critical in order to have a deeper understanding of the effects that human activities have on these ecosystems, and remote sensing techniques have widely been used with this purpose (Kerr 2003; Röder and Hill 2009). A great number of studies have used remote sensing techniques to calculate indices related to vegetation cover and monitor the stage of degradation of plant communities (Pickup *et al.* 1994; Hill *et al.* 1998; Harris and Asner 2003). However, the applicability of most of these methods is restricted to arid rangelands,

and there is a lack of techniques that can be applied to Mediterranean environments. Röder *et al.* (2007) performed the first attempt to solve this problem by using a cost surface modelling approach, which integrated the most important determinants of the spatial distribution of livestock, and afterwards used woody vegetation cover to infer the grazing gradient. This approximation, however, does not deal with the effects that grazing have on the herbaceous layer, despite its evident importance in grazing management.

Some difficulties have to be faced when studying the herbaceous layer in these systems. First, Mediterranean environments are highly heterogeneous (Thornes 2007), complicating the process of disentanglement and quantification of the relative importance of physiographical and grazing management variables. Second, the intimate mixing between patches of woody and patches of herbaceous vegetation typical of Mediterranean environments (Kuemmerle *et al.* 2006) further complicates the issue of developing a technique to monitor herbaceous cover. However, these problems can be solved thanks to the recent development of machine learning techniques (Elith et al 2006,2008; De'ath 2007; Crisci *et al.* 2012) that allow to analyse the complex relationships between environmental and management variables and to the availability of remote sensing information provided by very high resolution satellites.

1.6 THESIS OUTLINE

Because of the link between grazing and Mediterranean ecosystems, the consequences of the process of grazing intensification and the associated grazing abandonment have been identified as an issue that deserves further research (Peco *et al.* 2005,2006; de Bello *et al.* 2005,2006; Golodets *et al.* 2009b; Tárrega *et al.* 2009). The motivation of this thesis is to describe the effects of grazing intensification/abandonment on the composition, functioning and persistence of these grasslands, specifically focusing on Mediterranean dehesas as one of the most representative examples of these ecosystems.

Although there are great differences in species composition between grazed and ungrazed areas, the mechanisms determining these differences are still far from being unveiled. A recent study has shown that the different germination responses of increaser and decreaser species under changes in light conditions caused by grazing could be one of the determinants of these compositional differences (Dobarro *et al.* 2010).

CHAPTER 2 explores the effects that cattle dung leachates have on the germination of species with different responses to grazing. In order to do that, seeds and seedlings of seven confamilial pairs of species with different responses to grazing were exposed to different concentrations of cattle dung leachates. The initial hypothesis was that increaser species can germinate and/or establish better than decreasers in dung-generated environments.

CHAPTERS 3, 4 and *5* focus on the influence that grazing intensification and abandonment have on the diversity of Mediterranean grasslands. Specifically, *CHAPTER 3* deals with the changes in species richness and functional trait diversity produced after grazing abandonment. The paper presented in that chapter also analyses the impact of change of use on other vegetation descriptors (floristic composition and weighted trait average for individual traits) and the environment (soil fertility, bare soil cover and litter cover), integrating this information to provide an overall assessment of the mechanisms by which land use changes influence the system, and the consequences of these changes for its functions.

The effects of resource limitation and disturbance on plant ecological strategies and community assembly processes are increasingly well known. However, there is a lack of work examining how temporal variation in resource availability interacts with spatial abiotic stress gradients and disturbance to influence assembly processes in plant communities. The study presented in *CHAPTER 4* uses functional diversity for key plant ecological strategy traits to explore how temporal and spatial variation in water availability interact with grazing disturbance to influence assembly processes in Mediterranean grassland communities.

Rainfall is predicted to decline in the Mediterranean Basin, as well as in many of the areas of the world where grazing is significant (Christensen *et al.* 2007). Therefore, it is necessary to achieve a deeper understanding of the response of plant communities to the interaction between grazing and climate. In *CHAPTER 5*, the effects of interannual and topographical variations in water availability on the taxonomic and functional diversity responses to grazing are examined at different spatial scales. A strong emphasis is put on how the relationship between functional and taxonomical diversities is affected by grazing and on whether water availability modulates this effect.

CHAPTERS 6 and *7* deal with the consequences of grazing intensification at the landscape scale. *CHAPTER 6* presents a methodology to monitor grazing effects on herbaceous vegetation in highly heteroge-

neous Mediterranean grasslands. The proposed technique analyses the difference in photosynthetically active vegetation cover between spring and summer using spectral mixture analysis of very high spatial resolution images. Boosted Regression Trees are used to disentangle the relative influence that physiographic and grazing management related variables have on this difference. Eventually, an example illustrating the applicability of the methodology in a Mediterranean dehesa is provided.

CHAPTER 7 investigates the lack of regeneration of the tree layer of dehesas in relation to the abandonment of transhumant practices. In order to cope with the problem of the lack of regeneration of the trees in dehesas, several authors have proposed different management alternatives, such as the temporal or permanent abandonment of grazing and the reduction in the stocking rates. However, and despite temporal cessation of grazing has been recently suggested as an option to reverse the global decline in scattered trees (Dorrough 2005; Fischer *et al.* 2010), the effect of the recovery of non-continuous grazing practices such as transhumance has not been tested to date. In the presented manuscript, the effects that seasonal grazing associated with transhumant pastoralism have on the regeneration and vegetative condition of holm oaks are analysed by comparing different dehesas with contrasting grazing regimes (transhumant seasonal grazing vs. permanent grazing).

Finally, in *CHAPTER 8*, the findings of the preceding chapters are summarised and some lines for future research are proposed.

Chapter 2

Does cattle dung leachates concentration determine differences in germination responses of grazing increasers versus decreasers?

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Abstract

Although grassland species are usually classified as grazing increasers or decreasers, the response mechanisms to herbivore action in these two groups have yet to be clarified. One such action is dung deposition. The present study tested the hypothesis that increaser species perform more efficiently than decreasers during germination and/or establishment in the presence of dung leachates. A phytotron experiment was conducted on fourteen Mediterranean grassland species from five families, with an equal number of increaser and decreaser species chosen for each family to control for phylogeny. Seeds were germinated and grown in different concentrations of cattle dung leachate. We monitored germination percentages, median germination time and root length, measured five days after germination. To analyse the response and identify potential thresholds, we fitted piecewise mixed models using family/species as nested random factors. Differences in germination parameters between the two groups peaked in favour of increasers under intermediate leachate concentrations. Root growth was also negatively affected by leachates in decreaser species and promoted in increasers. Livestock dung deposition can increase the germination and colonising performance of increasers over decreasers, and may therefore be one of the mechanisms that explain the different species composition of grazed and ungrazed environments.

2.1 INTRODUCTION

Extensive grazing by large herbivores has a decisive effect on the composition and structure of herbaceous communities in Mediterranean grasslands (Noy-Meir *et al.* 1989; Peco *et al.* 2005). It plays a fundamental role in shaping and maintaining Mediterranean vegetation in different ways, through the consumption of leaves, flowers, fruits and roots, trampling and also through dung and urine deposition. These actions affect plant colonization and extinction rates in communities (Olff and Ritchie 1998), and act as a filter for their species. Many Mediterranean grassland species, including some endemics, are exclusive to grazed or non-grazed sites, with relatively few species found in both. Local-scale diversity can thus be encouraged by maintaining grazed and ungrazed areas in the same area (Verdú at al. 2000; Peco *et al.* 2006b; Shitzer at al. 2008; Bugalho *et al.* 2011).

Species are usually described as grazing increasers or decreasers, depending on their presence-absence response to grazing in a given zone. This classification is usually consistent, even at continental scales (Vesk and Westoby 2001). Many studies have explained these responses on the basis of mechanisms that differ between species, including tolerance to herbivory (Anderson and Briske 1995; del-Val and Crawley 2004,2005; Bonanomi *et al.* 2006; Wise and Abrahamson 2007), herbivore selectivity (Anderson and Briske 1995; Cingolani *et al.* 2005b), herbivore seed dispersal (Malo and Suárez 1995b; Will and Tackenberg 2008), plant-microbe interaction (Dormaar *et al.* 2002) and herbivore disturbance of the nutrient cycle (Peco *et al.* 2006b; Holdo *et al.* 2007; Tárrega *et al.* 2009). In recent years, several authors have tried to identify plant functional traits associated with grazing (Díaz *et al.* 2007a). Although some inconsistencies have been found, grazing generally favours annuals over perennials, short plants over tall plants, prostrate plants over upright plants, and those with stolons or a rosette form (see review in Díaz *et al.* 2007a).

However, few studies have attempted to experimentally quantify vegetation responses to different types of livestock action. Field experiments suggest that defoliation and trampling have relatively greater influence on the structuring of communities in grazed areas, while dunging has less effect (Kohler *et al.* 2004; Kohler *et al.* 2006; Dobarro 2009). Nevertheless, dung deposition and decomposition has been found to have a significant effect on the formation of patches of some plant

species (Dai 2000; Gillet *et al.* 2010). Experiments under controlled conditions have examined the different response by increaser versus decreaser species with respect to defoliation (del-Val and Crawley 2004 2005) and light intensity and spectral composition (Dobarro *et al.* 2010). Results from these experiments show that increasers tolerate defoliation better than decreasers (del-Val and Crawley 2004 2005), and increasers germinate less than decreasers in environments where the red/far red ratio indicates the presence of potential competitors (Dobarro *et al.* 2010). In addition, Leoni *et al.* (2009) have found a higher growth rate of increasers over decreasers.

In Mediterranean grasslands, which are mainly dominated by annual plants (Peco *et al.* 2006b), germination plays a decisive role in explaining species composition (Marañon 1998). Although it is acknowledged that dung deposition can have a major effect on seed dispersal (Malo and Suárez 1995b) and that there are great differences between species in their ability to survive and germinate after ingestion by herbivores (Peco *et al.* 2006a; D'hondt and Hoffmann 2011), the effect of dung leachates on seeds already present in the seed bank have not been tested before. Cattle dung is a major source of nutrients and phytotoxic compounds (Hoekstra *et al.* 2002), some of which affect plant germination and initial growth (Hillhorst and Karssen 2000; Farnsworth 2008; Berendse and Möller 2009; Luna and Moreno 2009). Breaking seed dormancy often depends on environmental conditions, and thus different species have different germination patterns depending on their environmental preferences (Vandelook *et al.* 2008). The differential effect of dung on the germination and establishment of different groups of species may therefore be one of the mechanisms that can explain the different species compositions in grazed and ungrazed environments. To date, no experimental study has considered the effect of dung on germination as a potential explanation for different species' response to grazing.

The present study aims to ascertain whether cattle dung leachate has different effects on the germination and root growth rates of species classified as increasers or decreasers. To do so, we measured germination and root length during the first days of seedling growth in several species in these two groups under different concentrations of cattle dung leachate. Our hypothesis was that species in the increaser group can germinate and/or establish better than decreasers in dung-generated environments.

2.2 MATERIAL AND METHODS

In June 2009, we collected fruits containing seeds of 14 herbaceous species (7 increasers and 7 decreasers) from five different families in grasslands in the Cerro de San Pedro area (40°43'N, 3°43'W, Madrid, Spain; see description in Peco *et al.* 2006b). To control for the effect of phylogeny, these species were separated into four confamilial pairs, the first of which was a grazing increaser and the second, a decreaser (see Table A1; *Brassica barrelieri* (L.) Janka paired with *Alyssum granatense* Boiss. and Reuter; *Spergularia purpurea* (Pers.) D. Don with *Silene scabriflora* Brot.; *Astragalus pelecinus* (L.) Barneby with *Vicia lathyroides* L. and *Poa annua* L. with *Micropyrum tenellum* (L.) Link) and 3 congeneric pairs (*Trifolium glomeratum* L. with *Trifolium strictum* L; *Plantago coronopus* L. with *Plantago lanceolata* L. and *Vulpia muralis* with *Vulpia ciliata* Dumort). Species classification was based on species abundance data from grazed and ungrazed plots in the same area. Only species with significant differences between grazed and ungrazed plots and present in more that 10% of the plots were used (see Table A1 for classification details).

In January 2009, we collected 5 kg of fresh dung from cows grazing in the study area (100g per dung pat, taken from 50 different pats). These dung samples were greenhouse-dried for two weeks, cut up into small pieces, randomly mixed and stored in paper bags in the lab. Dry dung was crumbled with a purée mixer and then weighed. Three litres of water per kg of dung were added and then mixed. This mixture was placed in a 9 cm diameter plunger coffee-maker and subjected to 0.2 Kg/cm^2 pressure for 30 seconds to produce a concentrated leachate (henceforth 25% extract). Given that the water content was similar to that reported for fresh cattle dung pats in previous studies (Dickinson *et al.* 1981; Hoekstra *et al.* 2002), the leachate concentration was similar to maximal dung leachate concentration in field conditions. Seeds in the field were assumed to be influenced by concentrations ranging from this maximum to zero, depending on the distance from dung pats. To simulate these conditions, three dilutions of this extract were produced with distilled water, in concentrations ranging from 25% (initial concentration), 10%, 5% to 1%. The nitrogen and carbon content of the 25% extract was defined by an element analyser (CHNS-932, LECO Corporation, Michigan, USA), while an ionic chromatograph (D600, Dionex Corporation, California, USA) was used for the anion and cation content (Table 2.1).

Twenty-five seeds from each species were placed on filter paper in 5 cm diameter Petri dishes, one per species, with a 1.1 g layer of vermi-

culite, then placed in 27.8x17.7x4.9 cm^3 aluminium trays, with 14 dishes, one per species, on each tray. Each tray was subjected to one of the five treatments involving 6 ml of cattle dung leachate in different concentrations and one control using distilled water.

Table 2.1. Chemical analysis of 25% dung leachate concentration.

Total N (mg/g)	Total C (mg/g)	Cl^- (ppm)	PO_4^{3-} (ppm)	SO_4^{2-} (ppm)	Na^+ (ppm)	K^+ (ppm)	NH_4^+ (ppm)
0.40	3.10	360.3	423.9	732.6	27.6	1695.8	9.3

Six replicates of each treatment were used, requiring 30 trays distributed randomly in two germination chambers (V-450, ASL s.a. Ibercex, Madrid, Spain). Replicates were kept in a 12 h (20°C) - 12 h (10°C) light/darkness and temperature regime. The moisture level in the trays was monitored visually every day, and maintained constant by adding distilled water when necessary. During the six-week experiment, the number of germinated seeds in each dish was monitored daily. At the end of the experiment, we calculated percentages of germinated seeds and T50 (time in days until 50% of germinations) for each dish.

Root length is one of the most widely used indicators of phytotoxicity in compounds that can influence plant germination and growth (Tiquia and Tam 1998; Hoekstra *et al.* 2002). To estimate length, germinated seeds were separated from the rest and placed in a dish with the corresponding treatment. Seedlings identified by transplant date were left to grow for five days, then image scanned to define root length after five days ($L5$) for at least four germinations per species and treatment using AutoCAD (2008). This measurement was impossible in three of the species (*A. pelecinus*, *V. lathyroides* and *T. strictum*), due to insufficient germinations.

Dung density in the field

Distance from the nearest dung pat probably influences the concentration of leachates affecting seeds in the soil. In order to assess the spatial relevance of this factor in the field, average distances from dung pats under grazing conditions were estimated by choosing a farm in the study area with an intermediate grazing pressure (0.51 cows/ha) defining twenty 50x50 m plots 100 m from each other along a south-north transect. Five points in each plot were chosen at random to measure the

distance from (1) the nearest detectable dung pellet and (2) the nearest dung pat of diameter >5 cm.

Statistical analysis

Linear mixed-effect models (Bates 2005) were used to to test the hypothesis that decreaser species are harmed in comparison with increasers in the presence of the dung extract. In order to test for consistent differences between species pairs, Germination difference (DGP) and $T50$ difference ($DT50$) indices were calculated as:

$$DGP_{ij} = \%\, germinated\ seeds\,(Increaser)_{ij} - \%\, germinated\ seeds\,(Decreaser)_{ij} \quad (2.1)$$

$$DT50_{ij} = T50\,(Increaser)_{ij} - T50\,(Decreaser)_{ij} \quad (2.2)$$

where i is the corresponding tray and j is the corresponding phylogenetic pair. For the response variables DGP and $DT50$, the explanatory variable was leachate concentration (continuous). Since species and families are not statistically independent (Felsenstein 1985), the variance across them was as a hierarchical effect. The nested effect of *Family/Species* was therefore used as a grouping factor with random effects, allowing the intercept to vary between groups. This type of model facilitates estimates of the percentage of total variability due to inter-family variability (Crawley 2007). The same modelling scheme was used for L5, fitting two separate models: one for increasers and another for decreasers. *Leguminosae* family data were excluded from these analyses due to insufficient germinations.

To check whether the response variables showed a non-linear response, we fitted piecewise regressions which allowed us to define thresholds beyond which this response changed. Four models were designed for each of the response variables. In one we assumed a linear response to the treatment while the other three corresponded to piecewise regressions in which we assumed the existence of a threshold (U) at which the response changed (Toms and Lesperance 2003; Cottingham *et al.* 2005). These thresholds were made to coincide with the 1%, 5% and 10% concentrations. We then chose the model with the lowest Akaike Information Criterion score for each response variable (AIC= -2 * log-likelihood + 2 * number of parameters).

The models were expressed as:

$$RV = \beta_0 + \beta_1 * T, \qquad for \quad T < U \quad (2.3)$$

$$RV = \beta_0 + \beta_1 * U + \beta_2 * (T - U), \qquad for \quad T > U \quad (2.4)$$

Where RV is the considered response variable (DGP, DT50 or L5), β_0 is the intercept, T is the dung leachate concentration, U is the considered concentration threshold, β_1 is the regression coefficient for $T < U$ and β_2 is the regression coefficient for $T > U$. In the models that did not consider a threshold in the response, only equation 2.4 was used, extending it to the full range of leachate concentrations.

Linear and piecewise regressions were performed independently for each family to observe the influence of the treatment on DGP, $DT50$ and $L5$ in each one. In families with more than two species, these were grouped according to their response to grazing. The procedure used to define the models that best explained the observed data was similar to that used for the mixed effect models. All analyses were conducted using the R v2.12.1 statistical package (R Development Core Team 2009).

2.3 RESULTS

Germination percentages

The model with a threshold at 1% concentration yielded the lowest AIC value (Table 2.2). DGP increased considerably between the control and this threshold, but further increases in leachate concentration showed no significant effects on this parameter (Table 2.3; Fig. 2.1). The difference in the germination percentages between increasers and decreasers was not significantly different from zero in the control (Fig. 2.1), while statistically significant differences between the two groups of species were appreciated for the rest of the treatment levels, in which the species in the increasers group germinated more than the decreasers. The family factor explained 32.1% of the variability found in this parameter. The best model for the families *Brassicaceae*, *Caryophyllaceae* and *Poaceae* included a threshold (1%, 1% and 5% concentrations, respectively). For these three families, DGP increased along with leachate concentration below the threshold. Concentrations higher than the threshold had no significant effect on DGP for *Bassicaceae* and *Poaceae*, and reduced this parameter significantly for the pair of *Caryophyllaceae* family species. For the pair of species from the *Plantaginaceae* family, a significant increase in DGP throughout the range of concentrations was observed. No thresholds were found for the legumes, for which the leachate concentration had no significant influence on DGP.

Germination speed

Increaser species germinated faster than decreasers in the control. This parameter remained favourable for the increasers at all leachate concentrations. The model that best explained the data included a 5% concentration threshold. In the range between the control and the 5% threshold, the difference in $T50$ between increaser and decreaser species rose significantly when the leachate concentrations were raised, while at higher concentrations, no effects of increased concentration were noted (Table 2.3, Fig. 2.2). The family factor accounted for 41.4% of the variability found in $DT50$.

The piecewise regressions for each family (Table 2.2) confirmed the existence of critical thresholds in the response by $DT50$ to the leachate concentration in *Brassicaceae* (10%), *Caryophyllaceae* (5%) and *Plantaginaceae* (5%). In these families, $DT50$ underwent a significant reduc-

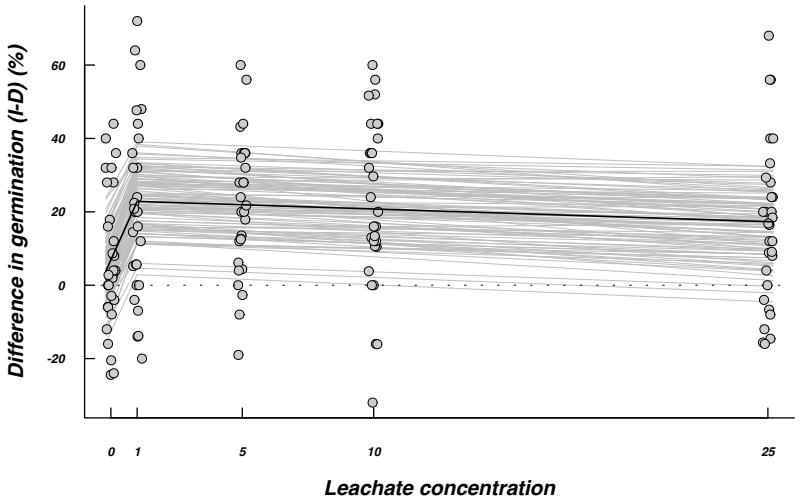

Figure 2.1. Effect of dung leachate concentration on difference in germination percentages of increasers and decreasers (DGP), analyzed by a piecewise regression with a 1% concentration threshold. The solid lines shows the best-fit regression. Thin lines represent 100 random draws indicating uncertainty in the fitted regression.

tion for leachate concentrations between the control and their respective threshold, due to a reduction in decreaser germination speed. Concentrations above the thresholds showed no significant effect on $DT50$. For the *Poaceae* family, $DT50$ declined linearly throughout the range of concentrations. Treatment had no significant effect on species in the *Leguminosae* family.

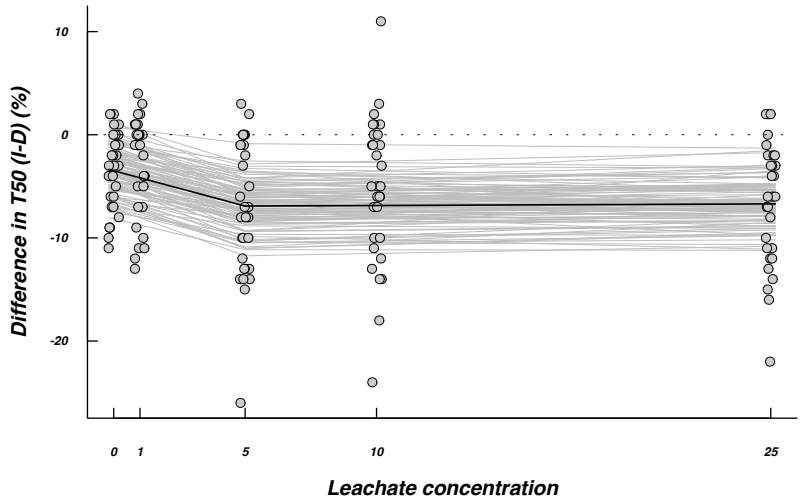

Figure 2.2. Effect of dung leachate concentration on difference in T50 of increasers and decreasers ($DT50$), analyzed by a piecewise regression with a 5% concentration threshold. The solid lines shows the best-fit regression. Thin lines represent 100 random draws indicating uncertainty in the fitted regression.

Root length

The model with a linear response to leachate concentration yielded the lowest AIC value for increaser species, while the model chosen for decreasers was the one with a threshold at the 10% concentration (Table 2.2). Root length in decreaser species was affected negatively by successive increases in leachate concentration below the threshold and remained unaffected afterwards. In contrast, higher leachate concentrations produced longer roots for increaser species (Fig. 2.3).

Due to insufficient germination, this variable was not analyzed for the species in the *Leguminosae* family (Table A3). In all other families except for *Plantaginaceae*, the relative root length of decreasers was diminished in comparison with increasers (Table 2.3).

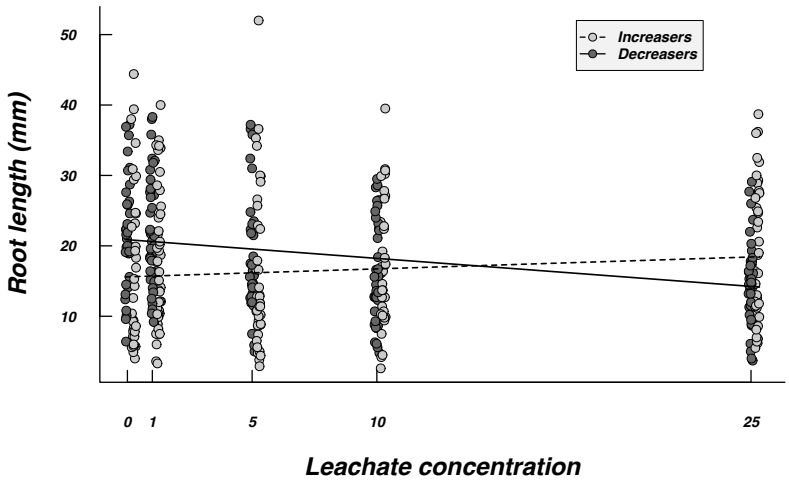

Figure 2.3. Effect of dung leachate concentration on root length after 5 days for increaser (analyzed by linear regression) and decreaser species (piecewise regression with a threshold at 10% concentration). The graph has been corrected to avoid overlapping points and facilitate its interpretation, slightly raising the values of the increaser treatment.

Distance from nearest dung pat

The median distance from the nearest dung pellet was 11.70 cm, and most of the sampled points were less than 20 cm. The median distance from the nearest dung pat measuring more than 5 cm was 74.2 cm, with very few cases more than 150 cm away (Fig. 2.4).

2.4 DISCUSSION

The main purpose of this study was to ascertain whether increaser and decreaser species behave differently in the presence of dung leachates. As expected, the difference between the germination percentages of increasers and decreasers grew considerably under the influence of leachates in comparison with the control. In the absence of treatment, increasers germinated faster than decreasers, with differences increasing for leachate concentrations below 5%. Root growth was also negatively affected by leachates in decreaser species and promoted in increasers.

Dung components can enhance or hinder seed germination performance. Previous studies have shown that higher nitrogen availability can stimulate germination in some species (Luna and Moreno 2009) and interact positively with light (Vandelook *et al.* 2008). Increased levels of

Table 2.2. AIC (Akaike's Information Criterion) values for the linear models and piecewise models with thresholds at different dung leachate concentrations (no threshold, 1%, 5% and 10%) . Response variables were: difference in germination percentage (DGP), difference in $T50$ ($DT50$), root length after 5 days for increaser species (Increaser $L5$) and root length after 5 days for decreaser species (Decreaser $L5$). Bold figures show the smallest AIC.

Family	Response variable	Linear model	1%	5%	10%
MIXED EFFECTS MODELS					
	DGP	1311.20	**1296.22**	1303.09	1306.23
	$DT50$	935.93	936.90	**930.47**	935.67
	Increaser $L5$	**1345.58**	1346.92	1346.86	1347.46
	Decreaser $L5$	1292.36	1293.78	1290.58	**1289.03**
MODELS FOR EACH FAMILY					
BRASSICACEAE	DGP	245.15	**243.02**	245.21	246.04
	$DT50$	175.41	177.41	175.77	**172.24**
	Increaser $L5$	**146.77**	148.42	148.34	148.03
	Decreaser $L5$	274.05	272.60	258.20	**258.10**
CARYOPHYLLACEAE	DGP	274.39	**266.01**	275.94	276.20
	$DT50$	176.26	173.69	**173.16**	174.42
	Increaser $L5$	229.93	**226.75**	230.89	231.75
	Decreaser $L5$	224.61	220.70	**218.84**	221.24
LEGUMINOSAE	DGP	**506.60**	508.55	508.29	507.90
	$DT50$	**355.87**	357.26	357.76	357.81
PLANTAGINACEAE	DGP	**250.78**	252.74	251.98	252.19
	$DT50$	203.56	204.39	**200.45**	205.55
	Increaser $L5$	**243.33**	244.29	245.18	245.24
	Decreaser $L5$	216.97	**216.40**	218.71	217.14
POACEAE	DGP	516.74	510.51	**507.00**	509.82
	$DT50$	**365.07**	366.70	366.93	367.07
	Increaser $L5$	691.59	692.97	**690.28**	692.96
	Decreaser $L5$	**576.70**	578.52	577.23	576.91

available nitrogen can be experienced by seeds as an indicator of a reduction in the number of plants competing with them for nutrients (Hilhorst and Karssen 2000; Fenner and Thompson 2005). On the other hand, seed germination and root growth can be delayed in the case of seedlings in contact with dung due to phytotoxicity associated with some of its components (Tiquia and Tam 1998; Hoekstra *et al.* 2002). Our results show that in the case of decreasers, the final balance of dung leachate input is a significant reduction in germination percentages and speed, as well as a reduction in their root length in comparison with increasers (Tables A2

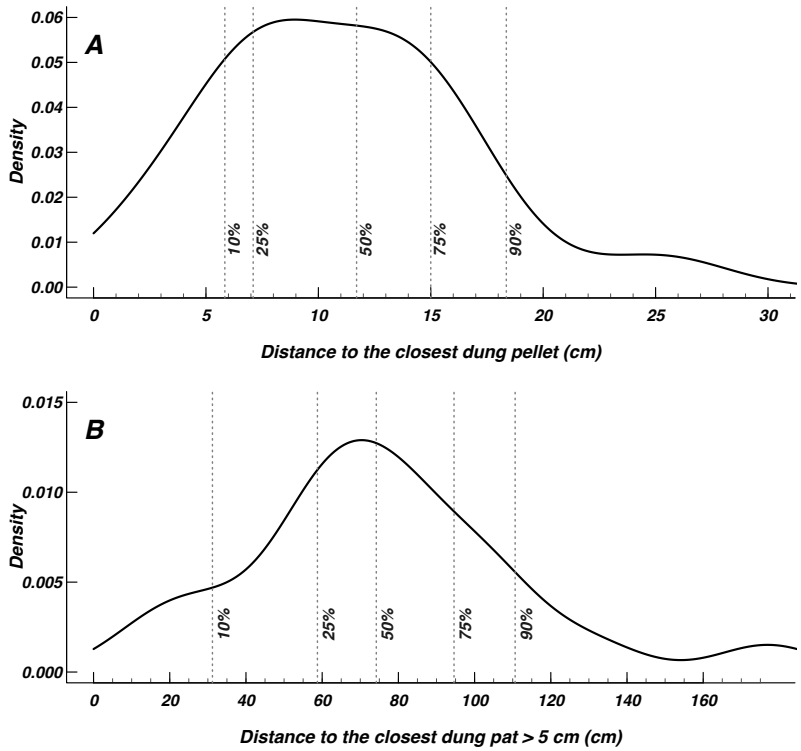

Figure 2.4. Probability density functions of (A) distance from random points to the nearest dung fragment and (B) distance from random points to the closest dung pat of diameter $>$ 5 cm. Quantiles for 10%, 25%, 50%, 75% and 90% probabilities are shown for each curve.

and A3). This suggests that the increaser species seeds and seedlings might tolerate dung leachate environments better than decreasers, with significant implications on the relationship between the colonising ability of both groups of species.

However, responses in germination percentage and speed were not linear, and we identified concentration thresholds beyond which the germination rate was not affected. Results revealed mechanisms that inhibit decreaser germination in the presence of low dung concentrations. This non-linear behaviour concurs with the results of previous studies aimed at identifying the determinants of different responses to grazing by increasers and decreasers. Del-Val and Crawley (2005) found a threshold above two cuts in responses to the number of defoliation episodes. Similarly, Dobarro *et al.* (2010), who examined germination response to light intensity and spectral composition by species with different responses to grazing, found a non-linear response by decreasers to variations in the red/far red proportion of incidental light. They also provided evidence

Table 2.3. Coefficients (mean ± standard error) and R^2 of the models with the lowest AIC values for difference in germination percentage (DGP), difference in T50 ($DT50$) and root length after 5 days ($L5$) for the mixed effects models and for each family.

Family	Response variable	U	β_0	β_1	β_2	R^2
MIXED EFFECTS MODELS						
	DGP	1%	6.80±6.38	**15.95±3.76*** **	0.23±0.17	
	*DT*50	5%	-3.49±1.93	**-0.68±0.21** **	0.01±0.06	
	Increasers *L*5	∼	**15.96±3.33*** **	**0.11±0.05***	∼	
	Decreasers *L*5	10%	**22.88±1.80*** **	**-0.57±0.14*** **	-0.07±0.10	
MODELS FOR EACH FAMILY						
BRASSICACEAE	*DGP*	1%	**-12.96±5.24***	**13.13±6.22***	0.05±0.28	0.11
	*DT*50	10%	0.52±1.72	**-0.68±0.20** **	0.01±0.14	0.33
	Increasers *L*5	∼	**8.69±1.09*** **	**0.72±0.07*** **	∼	0.77
	Decreasers *L*5	10%	**22.97±1.53*** **	**-1.23±0.17*** **	-0.03±0.12	0.62
CARYOPHYLLACEAE	*DGP*	1%	10.15±7.69	**30.29±9.43** **	**-1.82±0.42*** **	0.40
	*DT*50	5%	**-8.81±1.36*** **	**-0.90±0.38***	-0.11±0.11	0.11
	Increasers *L*5	1%	**5.89±1.01*** **	**2.88±1.23***	-0.04±0.06	0.13
	Decreasers *L*5	5%	**25.03±2.08*** **	**-2.06±0.59** **	-0.14±0.16	0.40
LEGUMINOSAE	DGP	∼	**10.03±2.77*** **	-0.14±0.22	∼	<0.01
	*DT*50	∼	**12.57±2.82*** **	-0.15±0.20	∼	0.01
PLANTAGINACEAE	*DGP*	∼	**32.29±3.64*** **	0.53±0.29	∼	0.07
	*DT*50	5%	**-5.82±2.14***	-1.24±0.60*	-0.36±0.17	0.11
	Increasers *L*5	∼	**13.55±1.14*** **	0.05±0.09	∼	<0.01
	Decreasers *L*5	1%	**16.89±3.80*** **	7.24±4.71	-0.19±0.19	0.02
POACEAE	*DGP*	5%	**7.64±3.80***	**4.09±1.08*** **	-0.36±0.31	0.18
	*DT*50	∼	-1.66±0.85	**-0.15±0.07***	∼	0.06
	Increasers *L*5	5%	**25.02±2.03*** **	-1.05±0.56	0.15±0.16	0.02
	Decreasers *L*5	∼	**21.38±1.05*** **	**-0.19±0.08***	∼	0.05

U: concentration threshold of the model with the lowest AIC score (symbol ∼ indicates a model with a linear response). β_0: intercept. β_1: regression coefficient for treatment concentrations below U. β_2: regression coefficient for treatment concentrations above U. *, P<0.05; **, P<0.01; ***, P<0.001.

of faster germination by increasers than decreasers, a feature confirmed by this study.

The low concentrations at which thresholds are found suggest that the effects on germination do not depend on the leachate concentration so much as its mere presence or absence. This kind of response might be explained by greater pathogen activity in the leachates environment, affecting the two kinds of species in different ways. Other authors have reported differences in sensitivity to fungal attack between different species (Leishman *et al.* 2000a; Schafer and Kotanen 2004) and variations in seed mortality amongst habitats as a result of these attacks (Blaney and Kotanen 2001), but more studies are needed to clarify this aspect.

Competition for nutrients is one of the fundamental processes that can be affected by variations in relative root length between the two groups of species and higher nutrient levels can increase the ratio between the aerial part and the roots (Poorter and Nagel 2000). Although the present study did not measure the aerial part of the seedlings or assessed the relationship between treatment concentration and resource distribution within the seedlings, the above-mentioned effects on root growth can have important consequences for seedling survival. Smaller root size in decreasers may significantly affect the drought resistance of seedlings of these species, given that longer roots facilitate access to more reliable water (Leishman and Westoby 1994; Leishman *et al.* 2000b). This aspect is probably relevant in the field, because droughts following the first autumn rains, immediately after germination, are a major factor in seedling mortality in Mediterranean environments (Espigares and Peco 1995; Hamilton *et al.* 1999).

The proportion of variability explained by the family factor in the mixed effect models seems to show that there is a correlation between the members of the same family in the study parameters, justifying the choice of species from a single family with different responses to grazing in order to control for the effect of phylogeny. Nevertheless, the results for the different families reflect responses by two or four species at the utmost, and extrapolations of the observed behaviour to the rest of the species in each family should proceed with caution. Interspecific differences in responses to grazing peaked at different leachate concentrations within families. This is not surprising in the light of the considerable variability between families in the three analysed parameters. However,

most of the observed responses are consistent with the hypothesis that dung leachate favours increaser species in comparison with decreasers.

The abundance of one of the increaser species, *A. pelecinus*, is known to greatly increase in plant communities after dispersal via herbivore dung (Malo and Suarez 1995a). The lack of effects by leachates on the analysed parameters in this and the other legumes in this family may be due to their seed coat impermeability, which prevented their penetration by the treatment (Baskin and Baskin 2001). All studied species were annuals, with the exception of *P. lanceolata*, which can behave as an annual or a perennial. Germination may therefore have less influence on this species' grazing response than the others. Nevertheless, its germination performance was reduced by the dung leachate (Table A2), suggesting that its decreaser response to grazing could be due to a reduction in its ability to colonize grazed areas. Besides the previously cited *A. pelecinus*, Malo and Suarez (1995b) found that increasers *P. coronopus*, *P. annua*, *S. purpurea*, *T. glomeratum* and *V. muralis* and the decreasers *M. tenellum* and *V. ciliata* all germinated in cattle dung, while decreasers *V. lathyroides* and *A. granatense* did not, despite their abundance in the sampled communities. Our study suggests that the ability to germinate under high concentrations of dung leachate is a factor that should be taken into account along with established aspects such as gut passage survival.

The detected effect of dung on grassland floristic composition is supported by previous studies which have emphasized the importance of soil fertilization (Dai 2000), lower grazing intensity around dung pats (Gillet *et al.* 2010) and dung-borne seed dispersal (Malo and Suárez 1995b), among others. The present study adds a new factor for consideration: the effects of dung leachates on the regeneration niche of herbaceous species, which are probably significant in both time and space in grazed areas, given that dung pats can take a long time to decompose in the field (Dai 2000), and that any given site is likely to be affected by cattle dung (Bakker and Olff 2003), as confirmed by the small average distances from the nearest pat (Fig. 2.4). Nevertheless, further research into this aspect is needed to confirm these responses under field conditions and their relative importance.

The effects of herbivores on environments where herbaceous plants germinate and grow seem to play a decisive role in the species composition of grassland communities. The present paper shows that, under laboratory conditions at least, the effect of dung deposition is a major factor in the germination, establishment and growth of species with dif-

ferent responses to grazing, which should be studied in more depth in natural ecosystems. The reduction of decreaser species' germination indices and root growth in the presence of dung leachates can help to explain the lower frequency or even the absence of these species in grazed environments.

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2.6 SUPPORTING INFORMATION

Additional supporting information for this article:

Table A1. Summary of the results of previous studies used to determine the increaser or decreaser response to grazing of each species.

Table A2. Mean ± Standard error of germination percentage and $T50$ for the different treatments and species used.

Table A3. Mean ± Standard error of root length after five days for the different treatments and species used.

Table 2.4. Table A1. Summary of the results of previous studies used to determine the increaser or decreaser response to grazing of each species. For each species and study the percentage of plots of each grazing level (G: grazed areas; UNG: ungrazed areas) in which the species was recorded is shown. Only studies in which the species is present in at least a 10% of the plots are shown.

Family, Species, and response to grazing		Peco *et al.* 2005[1]		Peco *et al.* 2006[2]		Carmona, 2012[3]	
		G	UNG	G	UNG	G	UNG
BRASSICACEAE							
Brassica barrelieri	I	∼	∼	∼	∼	41	3
Alyssum granatense	D	1	10	∼	∼	0	12
CARYOPHYLLACEAE							
Spergularia purpurea	I	61	10	82	4	46	0
Silene scabriflora	D	0	27	0	40	0	44
LEGUMINOSAE							
Astragalus pelecinus	I	18	0	36	0	10	0
Vicia lathyroides	D	0	35	0	22	2	33
Trifolium glomeratum	I	52	8	88	30	50	0
Trifolium strictum	D	∼	∼	4	28	4	30
PLANTAGINACEAE							
Plantago coronopus	I	11	0	38	0	63	0
Plantago lanceolata	D	∼	∼	2	32	21	74
POACEAE							
Poa annua	I	∼	∼	56	0	92	0
Micropyrum tenellum	D	1	24	0	20	∼	∼
Vulpia muralis	I	41	34	68	10	87	20
Vulpia ciliata	D	15	29	6	38	8	21

Symbol ∼ indicates that the species was not present in at least a 10% of the plots in the corresponding study. [1] Five sites in grazed and five in ungrazed areas surveyed in spring 1996. At each site, 20 quadrats (20x20cm) were sampled. [2] 10 sites in grazed and 10 in ungrazed areas sampled in spring 2000. At each site, 10 quadrats (20x20cm) were sampled. [3] 33 sites in grazed and 34 in ungrazed areas sampled in spring 2009. At each site, three quadrats (20x20cm) were sampled.

Table 2.5. Table A2. Species selected for the experiment, classified according to family and response to grazing (I: Increaser; D: Decreaser). Mean ± Standard error of germination percentage and time to 50% of germination (T50) for the different treatments and species (control, C; 1,5, 10 and 25% leachate concentration).

Family, Species, and response to grazing		Germination (%)					T50 (days)				
		C	1%	5%	10%	25%	C	1%	5%	10%	25%
BRASSICACEAE											
Brassica barrelieri	I	31.76±4.05	30.27±5.35	29.07±7.24	26.99±2.93	42.73±7.25	2.83±0.48	3.67±0.56	2.50±0.22	3.50±0.56	5.17±1.68
Alyssum granatense	D	44.67±2.62	30.69±2.18	28.05±7.27	26.01±4.11	41.33±3.82	3.50±0.34	2.83±0.40	4.17±1.17	10.33±2.04	11.17±2.71
CARYOPHYLLACEAE											
Spergularia purpurea	I	60.00±6.69	82.67±5.13	62.00±7.50	69.33±8.80	66.00±6.09	2.17±0.17	2.67±0.21	3.00±0.45	4.17±0.87	4.33±1.02
Silene scabriflora	D	51.67±2.52	34.67±2.86	38.67±2.86	51.33±6.06	68.00±3.72	9.50±0.56	13.00±1.10	15.33±0.95	16.67±2.38	14.83±0.98
LEGUMINOSAE											
Astragalus pelecinus	I	6.03±1.71	7.39±1.61	3.39±1.64	7.36±1.21	6.00±1.37	8.40±4.49	3.40±1.60	7.33±5.84	8.00±4.65	5.17±2.36
Vicia lathyroides	D	8.95±2.55	11.61±3.91	8.22±3.39	7.60±2.18	8.37±1.96	13.60±4.27	23.00±7.34	30.75±2.29	24.20±7.32	19.00±4.58
Trifolium glomeratum	I	26.00±7.06	34.67±5.72	25.33±3.68	16.00±3.27	26.00±1.71	2.67±0.21	4.00±0.58	4.17±0.70	3.00±0.63	3.83±0.70
Trifolium strictum	D	4.67±1.91	4.00±1.46	2.67±0.84	5.33±1.33	8.00±1.03	16.00±6.39	12.50±5.85	9.25±2.50	10.60±2.91	6.83±2.32
PLANTAGINACEAE											
Plantago coronopus	I	64.67±2.17	48.00±8.52	54.00±6.18	61.33±5.63	66.00±7.50	5.33±1.58	6.17±2.21	4.00±0.86	12.50±3.94	6.17±1.30
Plantago lanceolata	D	33.33±5.81	18.67±5.02	15.47±2.23	21.33±5.43	22.00±4.70	10.00±0.97	10.67±1.71	19.00±2.46	14.83±1.83	10.83±1.28
POACEAE											
Poa annua	I	59.33±5.10	51.33±5.41	59.33±2.40	56.00±4.38	54.87±5.21	6.33±0.33	5.67±0.21	6.67±0.56	6.83±0.48	8.67±0.33
Micropyrum tenellum	D	56.00±3.43	27.39±6.61	27.64±4.55	22.06±5.70	33.33±5.53	7.50±0.43	6.17±0.98	10.50±1.61	11.67±2.03	18.33±3.13
Vulpia muralis	I	88.58±4.78	87.33±4.06	98.67±0.84	94.67±2.86	91.33±3.49	7.17±0.40	7.83±1.05	7.33±1.05	11.67±0.67	13.17±1.08
Vulpia ciliata	D	85.81±5.67	84.34±3.53	81.05±3.77	76.82±6.85	75.05±3.74	10.67±2.17	8.33±1.33	10.50±1.34	12.17±1.45	14.33±2.38

Table 2.6. Table A3. Species selected for the experiment, classified according to family and response to grazing (I: Increaser; D: Decreaser). Mean ± Standard error of root length after 5 days for each treatment applied to each species (control, C; 1, 5, 10 and 25% leachate concentration). Superscripts indicate the number of samples.

Family, Species, and response to grazing		Root length after 5 days (mm) C	1%	5%	10%	25%
BRASSICACEAE						
Brassica barrelieri	I	8.04±0.52^{7}	10.73±1.4^{7}	^{0}	14.17±4.36^{4}	27.19±1.20^{7}
Alyssum granatense	D	22.45±2.05^{10}	23.11±1.5^{7}	14.36±0.68 ^{7}	10.99±1.18 ^{10}	9.96±0.97 ^{10}
CARYOPHYLLACEAE						
Spergularia purpurea	I	5.93±0.37 ^{9}	9.17±1.2 ^{9}	8.99±1.07 ^{9}	8.47±1.21 ^{8}	9.90±1.09 ^{9}
Silene scabriflora	D	25.77±3.38 ^{6}	20.52±3.4 ^{6}	14.55±2.24 ^{6}	13.19±1.25 ^{7}	11.36±1.90 ^{8}
LEGUMINOSAE						
Trifolium glomeratum	I	14.14±1.49 ^{7}	13.30±1.9 ^{7}	22.40±1.75 ^{7}	11.55±2.54 ^{6}	10.68±2.39 ^{8}
PLANTAGINACEAE						
Plantago coronopus	I	11.78±1.76 ^{6}	14.65±1.8 ^{8}	14.02±2.21 ^{9}	14.35±0.75 ^{8}	14.74±2.34 ^{8}
Plantago lanceolata	D	16.70±4.59 ^{6}	26.70±3.5 ^{8}	24.75±6.25 ^{2}	14.28±3.93 ^{5}	21.09±1.61 ^{8}
POACEAE						
Poa annua	I	31.68±2.93 ^{9}	30.45±2.3 ^{10}	31.06±3.72 ^{9}	30.87±1.54 ^{7}	28.56±2.88 ^{9}
Micropyrum tenellum	D	19.10±1.96 ^{6}	26.71±1.5 ^{9}	31.52±2.63 ^{6}	25.80±1.02 ^{7}	18.50±3.59 ^{4}
Vulpia muralis	I	17.84±2.50 ^{8}	20.67±1.7 ^{11}	10.78±1.90 ^{13}	17.71±2.15 ^{8}	15.59±3.03 ^{7}
Vulpia ciliata	D	21.71±2.13 ^{11}	14.92±1.5 ^{11}	15.09±1.98 ^{10}	17.70±2.53 ^{8}	14.82±0.92 ^{12}

Chapter 3

Effects of grazing abandonment on functional and taxonomic diversity of Mediterranean grasslands

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Abstract

This paper analyses changes in species richness and functional trait diversity in response to grazing abandonment in Mediterranean grasslands, for five traits that are well connected to ecosystem function and community assembly: Plant Height, Specific Leaf Area, Seed Mass, Onset of Flowering and Growth Form. The impacts of land-use change on floristic composition and community-weighted traits were measured along with soil fertility, bare soil cover and litter cover. Results reveal changes in the community average value of all analysed plant functional traits and a loss of functional diversity after abandonment in two of them, which is not accompanied by a change in species richness, indicating a loss of functional groups and a potential effect on the ecosystem functioning.

Abandonment is accompanied by the disappearance of short species, prostrate or creeper growth forms and early flowering species, while tall species and graminoid forms increase, their greater inflammability predicting an increase in wildfire frequency and intensity. Seed Mass and Specific Leaf Area show a weak but significant signal, with abandonment favouring large-seeded species and small Specific Leaf Area. A decline in soil fertility after abandonment is also detected, probably related to the decreased nutrient recycling rate and litter decomposability.

3.1 INTRODUCTION

In Europe, many agrosystems with high natural values such as semi-natural grasslands, heathlands, arable steppes and agroforestry systems are undergoing complex changes in land use which threaten their biodiversity and the ecosystem services they provide. Most of the studies examining changes in species and functional diversity with respect to land use changes have focused on the impact of intensification processes (Dorrough and Scroggie 2008; Flynn *et al.* 2009; Laliberté *et al.* 2010), while the abandonment of traditional human activities, including extensive grazing, has received less attention (Castro *et al.* 2010; MacDonald *et al.* 2000; 2006; Pakeman and Marriot 2010; Peco *et al.* 2005).

Species diversity, usually measured as species richness, is the most widely studied component of biodiversity (Helm *et al.* 2006; Krewenka *et al.* 2011), although in recent years, there has been a proliferation of the focus on functional diversity (Díaz *et al.* 2007b; Flynn *et al.* 2009; Laliberté *et al.* 2010; Tscharntke *et al.* 2008). The use of species richness is justified by its ease of measurement and the assumption that this component of diversity is well correlated with ecosystem processes and properties, including resource use, biomass production and stability (Balvanera *et al.* 2006; Duffy *et al.* 2003; Ives *et al.* 2000; Mittelbach *et al.* 2001). Changes in species diversity should be regarded as an outcome of ecological assembly processes, not a causal driver of ecosystem function (Mayfield *et al.* 2010).

In contrast, functional diversity, which focuses on the functional traits of species in a community rather than taxonomic units, can be a more direct, mechanistic link to ecosystem processes than species richness (Díaz and Cabido 2001; McIntyre and Lavorel 2001; Petchey and Gaston 2006).

Land-use change can affect both species diversity and functional traits. According to the sampling effect hypothesis (SEH), trait states (or values) accumulate with successive additive random draws from a regional species pool, implying a positive relationship between species diversity and functional diversity (Huston 1997). However, changes in species diversity and functional trait diversity (ΔDSF) can in fact take different paths in response to land-use change, and species diversity- functional diversity are not necessarily correlated (Mayfield *et al.* 2006,2010). According to Mayfield *et al.* (2010), land-use change impacts upon community assembly processes, not species per se, and

changes in species diversity should be considered an outcome of ecological assembly processes.

Many attempts have been made to identify grazing-related plant traits, but few general patterns have emerged (see review in Díaz *et al.* 2007a). Basically, the only generalizations have dealt with the encouragement of species with prostrate habits and annuals over perennials, however even these responses seem to be context-dependent (Díaz *et al.* 2007a). One of the difficulties encountered in the search for relationships between plant functional traits and grazing at a global scale (Díaz *et al.* 2007a) is that this response depends on the precise level of grazing (Bullock 1996), the type of herbivory and its selective behaviour (Evju *et al.* 2009), productivity (de Bello *et al.* 2005; Pakeman 2004; Vesk and Westoby 2001) and the herbivory history (Díaz *et al.* 2007a; Milchunas and Lauenroth 1993). We must therefore define the rules of the response to grazing by the different plant traits for each climatic context and grazing history (Díaz *et al.* 2007a).

Dehesa grasslands (*Montados* in Portuguese) in the Mediterranean basin have a long history of grazing. The abandonment of long-term grazing does not seem to affect species richness (Peco *et al.* 2006), while the abandonment of cropping does so (Castro *et al.* 2010). Grazing abandonment produces an increase in the proportion of taller plants, high leaf dry weight, late flowering species and chamaephytes, as well as species with heavy seeds and fruits with adhesive structures (Peco *et al.* 2005). The vegetation in abandoned croplands becomes dominated by chamaephytes and nanophanerophytes, and there is an increase in Plant Height, Dry Leaf Matter Content and Seed Mass, while Specific Leaf Area (SLA) decreases (Castro *et al.* 2010). In addition to changes in the vegetation, changes in the environmental filtering due to abandonment have been documented (Peco *et al.* 2006). To date, however, no published studies have approached the impact of grazing abandonment on functional diversity in Mediterranean grasslands or the trajectory of species richness in relation to functional diversity (Mayfield *et al.* 2010).

The main goal of this study is to analyse changes in species richness and functional trait diversity (ΔDSF) in response to grazing abandonment in Mediterranean grasslands for five traits (Plant Height, Specific Leaf Area, Seed Mass, Onset of Flowering and Growth Form) that are well connected to ecosystem function and community assembly. The paper also analyses the impact of change of use on other vegetation descriptors (floristic composition and weighted trait average for individ-

ual traits) and the environment (soil fertility, bare soil cover and litter cover), integrating this information to provide an overall assessment of the mechanisms by which land use change influences the system, and the consequences of these changes for its functions.

3.2 METHODS

The study was conducted on the southern slopes of Sierra de Guadarrama (Central Spain) in a 15,000 ha area near San Pedro Peak (40°38'N, 3°70'E). The lithology is silica, predominantly gneis, Precambrian and Palaeozoic granite. The area had a gently rolling relief, mostly between 800 and 1000 m asl. The climate is continental Mediterranean (mean annual temperature: 13.2 °C; average annual rainfall: 550 mm at Colmenar Viejo station). The predominant soil type is Dystric cambisol.

The fields varied in size between 5 and 20 ha., with stone wall demarcations. Until the middle of the last century, all this area was used for low-density sheep and cattle grazing (around 0.2 to 0.3 GU/ha) which were moved between fields to make the most of the pastures. Some of these properties were still grazed by livestock, while others had not been grazed for more than 30 years.

Grassland and scattered trees (mainly *Quercus ilex* subsp. *ballota* and *Juniperus oxycedrus*) were the predominant vegetation types. The herbaceous layer mainly consisted of annuals, with a progressive increase in perennials with increasing soil moisture in water discharge areas and valley floors. We selected 19 sites that met the following requirements: *i)* on two environmentally similar adjacent properties, one in use and the other abandoned for at least 30 years, separated by a well-preserved stone wall that effectively prevents cattle movement between the two, *ii)* on dry slopes of less than 15%, *iii)* neither of the sites shared the same stone wall and *iv)* at least 500 m from the nearest town. This paired design permitted the analysis of land change use, independently of other environmental factors, and facilitated the use of more powerful statistical techniques.

Sampling

Two 10 x 10m plots were set one on grazed and one on abandoned site per pair, at least 5 m from the property wall. In every plot five 20 x 20

cm quadrats were placed, one in the center and the other four in each corner.

All plant species present in each quadrat were recorded in May 2002, yielding a frequency value between 0 and 5 for each species and plot, and a richness value for all five quadrats in the same plot. During the vegetation sampling, we also recorded the bare soil and litter cover in each quadrat using an ordinal scale, ranging from 0 to 5 (0: 0, 1: 1-12, 2: 12-25, 3: 25-50, 4: 50-75, 5: 75-100, intervals commonly used in vegetation studies).

In order to control for the possible effect of productivity on the vegetation response to grazing (de Bello *et al.* 2005; Pakeman 2004; Vesk and Westoby 2001), soil fertility was estimated by a bioassay with barley (*Hordeum vulgare*). Four soil cores were collected at random in each plot (5 cm diameter and 10 cm deep), air dried and sieved through 2 mm mesh. The soil samples from each plot were mixed and set in three pots (5 cm square and 6 cm deep), planted with eight barley seeds. The pots were kept in greenhouse conditions for four weeks, watered regularly to keep the soil moist. After germination, only two seedlings were left in each cell. The pots were relocated weekly to randomize the possible effect of position. After the bioassay, all plants were collected, cleaned and oven dried for 72 h. at 80 °C. We calculated the average dry biomass per plot, which was used as an estimator of the plot's soil fertility.

Five functional traits were measured in the study area, four quantitative (Plant Height, Specific Leaf Area (SLA), Onset of Flowering and Seed Mass) and one qualitative (Growth Form). Plant Height was defined as the distance from the plant base to the highest leaf (excluding reproductive tissue and protruding branches) in ten mature individuals from each species, set at least 25 m apart. SLA was defined as the ratio between leaf area and dry leaf weight, measured by collecting a leaf from each of the ten individuals previously used to measure Plant Height. We chose the youngest fully developed leaves, including the petiole. We scanned the hydrated and expanded leaves, and measured the area of each image with an image analyzer program (Leica Q500 Iw). The same leaves were weighted with a precision balance after oven drying at 60-80 °C for 24 h. Exceptionally, and in the case of small leaf species, we formed ten groups of 5 to 10 leaves for the surface and weight measurements.

Seed Mass was taken from Azcárate *et al.* (2002) in most cases. New measurements were also made for some species not included in the latter

study, using the same protocol (30 dry seeds per species), "*seed*" being defined according to Bekker *et al.* (1998).

Onset of Flowering was coded by month, from 1 (January) to 9 (September), and treated as a quantitative variable. Growth Form was treated as a qualitative variable, consisting of four categories: graminoid, erect, prostrate and rosette. Both traits were taken from specific bibliography on Iberian flora (Castroviejo 1986-1999; Valdés *et al.* 1987).

The paucity of some species made it impossible to obtain all five traits for all species recorded in the samples. Species that lacked at least one functional trait were deleted from the analysis of functional traits. All species affected by this problem showed a frequency of less than 10% in the quadrats, in both grazed and abandoned plots, while 89% of the presences identified in the grazed plots and 87% of those detected in the abandoned plots corresponded to species fully characterized by their functional traits.

Data analysis

The effect of abandonment on bare ground cover and litter cover was analysed using a sign test, due to the ordinal nature of the data collected. Soil productivity was analysed using paired t-tests. Each of the 19 locations was regarded as a sampling unit, and the two observations (grazed vs. abandoned) were treated as repeated measures. We also analysed the potential existence of a productivity gradient between sites using a rank correlation coefficient (Spearman) of the grazed plot's fertility in comparison with its non-grazed counterpart.

For the floristic composition analysis, a Multidimensional scaling (MDS) was built to synthesize the species frequency data of the 38 plots on a small number of axes. The analysis was based on a dissimilarity matrix of Euclidean distances between plots. To test whether the floristic composition differed between grazed and abandoned plots, repeated measures MANOVA were used, with the first two axes of the MDS as dependent variables and the paired grazed and abandoned plots as repeated measures. Floristic similarity between each pair of plots (grazed and abandoned) was also measured using the Jaccard index.

For each of the 38 plots, we calculated an aggregated plant trait value in the community for each of the five traits analysed. In the case of the quantitative traits, this was performed by averaging the log-transformed trait values of the component species on the site, weighted

by their frequency. The effect of abandonment on each trait (weighted trait average) was analysed with paired t-tests. For the qualitative trait Growth Form, the comparison was done for each of the trait states, taking the percentage of species corresponding to each one.

The functional diversity of the quantitative traits was calculated as the standard deviation of the frequency-weighted, $\log_e$-transformed trait values (Mayfield *et al.* 2010). For Growth Form, diversity was calculated as a Shannon index of the number of species with each trait state (Mayfield *et al.* 2010). Species richness was used as an indicator of species diversity. Mean response trajectory of the plots in relation to grazing abandonment was measured as the vector that connects the centroids of grazed and abandoned plots in the space of species richness and functional diversity for each analysed trait (see Mayfield *et al.* 2010). The significance of the differences in species richness and functional diversity between grazed and abandoned plots was examined by paired t-tests.

3.3 RESULTS

Soils in abandoned plots were less fertile than those in grasslands with on-going use, and their litter cover was greater. No significant differences were found in bare soil cover (Table 3.1). The sites were not ranked on a productivity gradient because the rank correlation coefficient of the fertility values for couples of grazed and ungrazed plots was not significant (Spearman r=-0.08, P=0.761).

In total, 218 species were detected in the samples (132 in grazed and 130 in ungrazed plots). Annuals predominated over perennials in both the grazed and the abandoned areas (80% and 76% of annuals respectively). Grazed and abandoned observations differed markedly in floristic composition (Fig. 3.1, repeated measures MANOVA: $F_{2,17}$=17.67, P$<$0.0001). The average similitude percentage (Jaccard index) between grazed and abandoned plots was 0.33$\pm$0.07.

The community aggregate trait values for plants differed between grazed and abandoned systems for three of the five analysed traits (Table 3.1). Vegetation in the abandoned grasslands showed a greater average Plant Height and a later Onset of Flowering. No differences were detected in Seed Mass or SLA. Numbers of graminoid and erect species increased, while rosettes and prostrates declined.

The mean response trajectories of ΔDSF from grazed to abandoned systems (Fig. 3.2) were short. The differences in taxonomic richness were

not significant (Paired-t-test=-0.59, P=0.5612) and significant differences in functional diversity were only found for two of the five analysed functional traits. Abandoned systems had lower functional diversity at the start of flowering (Paired-t-test= 2.73, P=0.014). The functional diversity of Growth Form also decreased (Paired-t-test=3.40, P=0.003). No differences between grazed and abandoned systems were observed for Seed Mass (Paired-t-test=- 0.088, P=0.165), Plant Height (Paired-t-test=-0.022, P=0.531) or SLA (Paired-t-test=-0.012, P=0.353).

3.4 DISCUSSION

The community aggregate trait values of Plant Height and Growth Form had a similar grazing-related response to other Mediterranean areas with a long history of grazing (Castro *et al.* 2010; Fernández-Alés *et al.* 1993; Hadar *et al.* 1999; Lavorel *et al.* 1999; Noy-Meir *et al.* 1989; Peco *et al.* 2005; Sternberg *et al.* 2000). Abandonment favoured taller species and graminoid or erect forms, while grazing favoured low stature species, as well as rosette or prostrate morphology. One possible mechanism that might explain this pattern is the removal of tall erect plants by herbivores, while small or prostrate species survive (Noy-Meir *et al.* 1989). Another mechanism could be an increased standing biomass and litter

Table 3.1. Mean and standard deviation of functional traits and soil variables in grazed and abandoned grasslands. t-statistics and P-value of respective paired t-tests are also shown (Sign test P-values in the case of bare soil cover and litter cover). N=19 for all cases. P-values lower than 0.05 shown in bold. SLA: Specific Leaf Area.

Variables	Grazed		Abandoned		t	P
	Mean	S.D.	Mean	S.D.		
Ln Onset of Flowering	1.47	0.06	1.53	0.08	-2.940	**0.009**
Ln Plant Height (*cm*)	1.81	0.13	2.01	0.23	-4.253	**0.0001**
Ln SLA (mm^2/mg)	3.16	0.07	3.14	0.07	1.158	0.262
Ln Seed Mass (*mg*)	-1.40	0.45	-1.29	0.41	-1.011	0.325
Growth Form (%)						
Graminoid	18.87	6.06	23.02	6.10	-2.460	**0.024**
Erect	41.81	7.35	48.37	6.41	-2.840	**0.011**
Rosette	29.41	7.04	21.24	6.89	3.693	**0.002**
Prostrate	9.90	6.67	7.37	6.89	3.372	**0.003**
Bare soil cover (%)	8.94	6.93	5.86	3.59	—	0.143
Litter cover(%)	2.96	3.29	7.70	4.30	—	**0.008**
Soil fertility (*mg*)	159.80	30.00	126.20	28.45	3.177	**0.007**

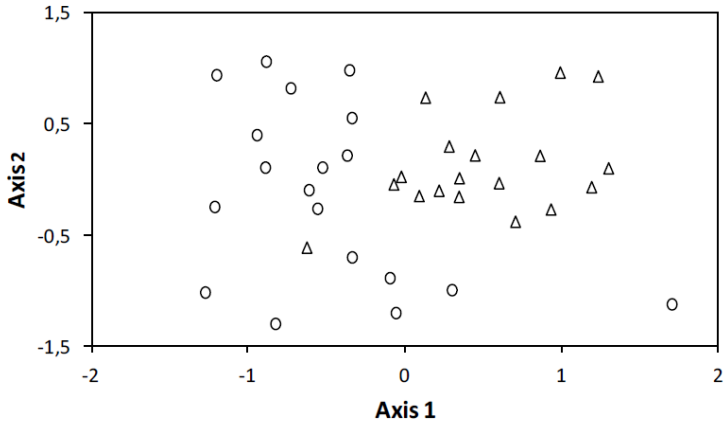

Figure 3.1. Representation of the vegetation plots in the space of the first two MDS axes. The position of the plots indicates their similitude in floristic composition. Final stress: 0.19. Open circles: grazed grasslands; open triangles: abandoned grasslands.

accumulation as a result of abandonment, accompanied by more competition for light, which would favours tall and erect species (Grime 2001). Our results also showed that grazing favours early flowering in these annual-dominated grasslands. This has also been found in other annual-dominated systems (Hadar *et al.* 1999), but not in perennial-dominated grasslands (Díaz *et al.* 1994; de Bello *et al.* 2005). Briske (1996) mentions early flowering as an avoidance strategy in relation to grazing.

Surprisingly, we did not find any effect of grazing abandonment on the community weighted average of Seed Mass. Most studies in Mediterranean environments relate grazed systems to light-seeded species (Castro *et al.* 2010; Lavorel *et al.* 1999; McIntyre and Lavorel 2001; Peco *et al.* 2005), although this relationship is not universal (Fernández-Alés *et al.* 1993; Landsberg *et al.* 1999). One possible cause of the discrepancy between previous results from Iberian dehesas and montados (Castro *et al.* 2010; Peco *et al.* 2005) and those in the present paper could be the dif-

ferent analytical methods used. The former papers analyzed the behavior of species with different Seed Mass (relative species cover of small, medium and large-seeded species) separately, finding that abandonment produces an increase in the relative cover of heavy seeded species, but no significant change in the other two species groups. This pattern was confirmed in a reanalysis of our data using this methodology, along with a significant increase in the relative cover of large-seeded species (Table 3.2).

Table 3.2. Mean and standard deviation of relative cover of each trait in grazed and abandoned grasslands, t-statistics and P-value of the respective paired t-tests are also shown. N=19 for all cases. P-values lower than 0.05 shown in bold. SLA: Specific Leaf Area.

Variables	Grazed		Abandoned		t	P
Seed Mass (mg)						
Light (<0.5)	12.00	8.48	10.80	7.17	0.496	0.626
Medium (0.5-1)	71.21	8.55	65.34	12.21	1.536	0.142
Heavy (>1)	16.79	9.12	23.86	13.16	-2.135	**0.047**
SLA (mm^2/mg)						
Low (<17)	19.93	8.99	21.45	11.41	-0.461	0.650
Medium-Low (17-24)	23.11	7.04	28.74	9.82	-2.228	**0.039**
Medium-High (25-30)	19.71	6.56	20.40	8.08	-0.255	0.802
High (>30)	37.26	9.55	29.41	11.26	2.564	**0.020**

We did not find any significant effect of grazing abandonment on the SLA community weighted average, whereas a reduction in SLA was found elsewhere (Castro *et al.* 2010; Cruz *et al.* 2010; Golodets *et al.* 2009a; Lindborg and Ericsson 2005). As in the case of Seed Mass, sometimes the detected trend was only for a fraction of SLA values. For example, the relative cover of species with intermediate SLA declined with abandonment (Peco *et al.* 2005). This trend was confirmed in the present study by the analysis of relative cover by species with different SLA, as in the case of weight. In this new analysis, we detected a significant trend by species with high SLA to diminish with abandonment, and species with intermediate-low SLA to increase (Table 3.2). The increase in community SLA with abandonment can be associated with a decrease in litter decomposability (Castro *et al.* 2010; Fortunel *et al.* 2009). However, the low response to abandonment by this character in quantitative analyses suggests that the effect of grazing on this trait is productivity dependent. Previous studies have found no consistent results for SLA in relation to grazing in arid environments (Vesk *et al.* 2004), or on mixed gradients

of productivity (fertility or rainfall) and grazing (Cingolani *et al.* 2007; Rusch *et al.* 2009).

Grazing abandonment produced a decrease in functional diversity for Growth Form and Onset of Flowering. Relative cover of species with rosette or prostrate growth forms fell drastically with abandonment, while cover of pre-existing species in the graminoid and erect groups increased. The loss of functional diversity in Onset of Flowering was due to the disappearance of early flowering species following abandonment. Given that species richness did not vary, this trend in ΔDSF suggests that at least in these two traits, abandonment involves the loss of functionally different species with potential consequences for ecosystem services (Mayfield *et al.* 2010). These results support the latter author's suggestion that the association of species and functional trait diversity can follow various trajectories in response to land-use change, and that functional and species diversity are not necessarily correlated.

Lower functional diversity in abandoned pastures can imply a decrease in the rates of ecological processes (Díaz and Cabido 2001; Naeem and Wright 2003). In fact, the abandoned systems in the study area were found to accumulate more non-decomposed biomass and have poorer soils, concurring with previous results (Peco *et al.* 2006) and indicating a possible loss of ecosystem services after abandonment and a decrease in the nutrient recycling rate.

3.5 ACKNOWLEDGEMENTS

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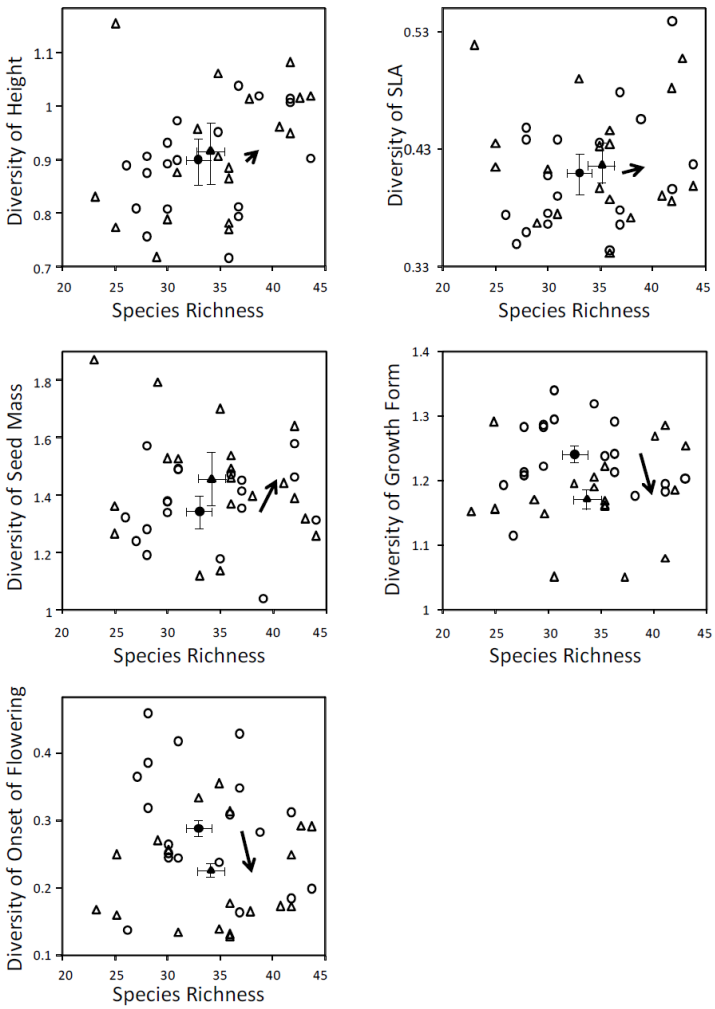

Figure 3.2. ΔDSF (changes in species richness and functional traits) patterns observed for the five functional traits considered in this study. Open symbols indicate species richness and trait state diversity for individual localities (circles for grazed and triangles for abandoned grasslands). Closed symbols indicate the mean species diversity and trait state diversity for grazed (circles) and abandoned grasslands (triangles). Error bars are 95% confidence intervals. Arrows in each plot illustrate the ΔDSF vector from grazed to abandoned grasslands. Height: Plant Height; SLA: Specific Leaf Area.

Chapter 4

Functional trait diversity indices reveal that interannual fluctuations in water availability promote diversity in semi–arid grasslands

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Abstract

This paper studies changes in assembly processes associated to interannual fluctuations in water availability along spatial gradients of resource availability and disturbance. Specifically, we focused on three key plant ecology strategy traits (Plant height, SLA and Seed mass). Plant communities from 67 sites under different grazing regimes (from heavy grazing to grazing abandonment) in wet and dry habitats were surveyed in a wet and a dry year. Community weighted mean and Rao quadratic entropy were calculated for each community. The use of appropriate null models allowed the estimation of pure measurements of functional richness and functional divergence, the two components of functional diversity with the highest power to detect changes in community assembly processes across environmental gradients.

The observed effects on vegetative traits (Height and SLA) were generally consistent with the expected shift from size-symmetric competition for belowground resources in dry years to size-assymetric competition for light in wet years. Functional richness patterns for Seed mass contrasted with those of the vegetative traits, revealing the simultaneous existence of trait convergence and divergence for different niche axes. Results support the role of pulses in water availability as a major determinant of plan diversity that reduces differences in average fitness between co-occurring species. The results also highlight the importance of the combined use of complementary measures of functional diversity based on individual traits rather than multiple combinations of traits when inferring assembly processes.

4.1 INTRODUCTION

Functional trait diversity is emerging as a powerful indicator of community assembly processes in plant communities (Mason *et al.* 2005, in press; Villeger *et al.* 2008; de Bello *et al.* 2010). Recent work has used functional diversity indices to reveal changes in assembly processes along spatial gradients of resource availability and disturbance. (e.g. Cornwell and Ackerly 2009; Pakeman *et al.* 2011; Mason *et al.* 2011,2012). However, it remains unclear how temporal variation in resource availability interacts with spatial gradients of stress and disturbance to influence assembly processes in plant communities.

One of the main factors affecting assembly processes in plant communities is water availability (e.g. Cornwell and Ackerly 2009). Soil moisture is highly related to primary productivity, as well as to the mineralization and uptake of soil nutrients (Rodriguez-Iturbe *et al.* 2001). Thus, increases in water availability are likely to produce a shift in the limiting factors for plant growth from soil resources to light, thus increasing the intensity of above-ground competition (Tilman 1988; Hautier *et al.* 2009). Whereas competition for belowground resources is size-symmetric, competition for light is size-asymmetric (Casper and Jackson 1997; Schiwinning and Weiner 1998; Berntson and Wayne 2000), a feature that has important implications for the assembly of communities. When below-ground competition prevails, the species with trait values close to the local optimum will be more abundant (Mouillot *et al.* 2007), resulting in reduced values of functional diversity (Spasojevic and Suding 2012; Mason *et al.* 2012,in press). On the other hand, when light is limiting, niche differentiation is required for species to coexist (Aikio 2004; Kohyama and Takada 2009). This should cause increased divergence in traits related to resource use and acquisition between coexisting species, leading to high functional diversity values for these traits (Stubbs and Wilson 2004; Spasojevic and Suding 2012; Mason *et al.* 2011).

Additionally, the changes in functional diversity associated with changes in water availability can be influenced by grazing (Carmona *et al.* 2012). Grazing and water stress are seen as convergent forces that select for similar trait values (Milchunas *et al.* 1988; Quiroga *et al.* 2010), which suggest that interannual changes in functional diversity associated to changes in water availability should be less evident in the most intensively grazed areas. Although disturbance (including grazing) has been considered as the most important cause of trait divergence (Grime

2006), recent studies have shown that grazing can cause trait convergence (Pakeman *et al.* 2011), but that its strength as a filter depends on productivity (Carmona *et al.* 2012).

In water-limited ecosystems, the relative abundance of plant species is strongly influenced by interannual fluctuations in water availability (Fernandez Illescas and Rodriguez-Iturbe 2003). Thus, variability in rainfall has been postulated as one of the mechanisms contributing to the maintenance of high species diversity in arid and semi-arid ecosystems (Chesson *et al.* 2004). Mediterranean grasslands experience high levels of interannual variability in rainfall, which causes great interannual differences in their species composition and diversity (Peco 1989; Espigares and Peco 1995; Peco *et al.* 1998; Carmona *et al.* 2012). Consequently, these grasslands are an adequate system to study the effects of the temporal variations in resource availability on assembly processes. Like many other European ecosystems, Mediterranean grasslands are experiencing a dual process of grazing abandonment in the less productive areas and intensification in the more productive or accessible ones (Stoate *et al.* 2009). This is threatening the diversity and ecosystem services provided by these systems (Sluiter and Jong 2006; Stoate *et al.* 2009).

Community structure encompasses complementary aspects that cannot be described with a single index (Díaz *et al.* 2007b). For example, communities located across environmental gradients can differ in their average trait values, in the dispersion of these values around the community average values, or in both aspects simultaneously. Consequently, the study of the variation in community assembly processes along environmental gradients requires the combined use of indicators of shifts in mean trait values and indicators of the patterns of trait convergence and divergence (Ricotta and Moretti 2011; Spasojevic and Suding 2012). The first aspect is taken into account with the examination of changes in average trait values across environmental gradients, which reveals the effect that habitat filtering has on the optimal trait values, thus indicating shifts in the traits of dominant species (Pakeman *et al.* 2011; Ricotta and Moretti 2011; Mason *et al.* 2012). Both abiotic stress tolerance or biotic filtering can be behind these changes, but in both cases they indicate the existence of equalizing fitness processes that enhance the abundance of species with trait values similar to a local optimum. (Chesson 2000; Mason *et al.* 2012; Spasojevic and Suding 2012). Additionally, trait convergence/divergence patterns are generally examined by comparing functional diversity indices with random expectations generated using null-

models (Gotelli and McCabe 2002). Among the different components of functional diversity (Mason *et al.* 2005), richness and divergence are the ones that can reveal the variation in assembly processes across environmental gradients (Mouchet *et al.* 2010; Mason *et al.* in press), while evenness seems to have a limited power to detect these changes (Mason *et al.* in press). Functional richness shows the influence of trait-based assembly processes on species occurrence, while functional divergence reveals effects on species relative abundances (Mason *et al.* 2012). Therefore, the combined use of functional richness, functional divergence and community average trait values is a powerful means of detecting changes in assembly processes along ecological gradients in a wide range of ecological contexts (Mouchet *et al.* 2010; Mason *et al.* in press).

This study uses functional diversity for plant traits to explore how temporal and spatial variation in resource availability interact with grazing disturbance to influence assembly processes in grassland communities. We examined community average trait values, functional richness and functional divergence for three key plant ecology strategy traits (SLA, plant height and seed mass; Westoby 1998) in two years with different water availability conditions caused by differences in rainfall. We compared these measures between two habitat types with contrasting levels of water availability situated along a gradient of grazing intensity. According to Mason *et al.* (2012), we tested the general prediction that diversity in traits related to resource use and acquisition should be positively associated with temporal and spatial variation in water availability. More specifically, we tested the following hypotheses: (1) due to the convergent effect of grazing and aridity, interannual changes in functional diversity indicators should be reduced in the highest end of the grazing gradient; (2) functional diversity for vegetative traits should be higher in wet habitats, where competition for light predominates, than in dry habitats, where below-ground competition predominates; and (3) differences in functional diversity between habitats should be reduced during the wet year, since the relative influence of light competition will be greater in dry habitats.

4.2 MATERIAL AND METHODS

Study area

The study area (5 x 4 km) is situated 35 km North of Madrid, in central Spain (40°38′ N; 3°70′ W; mean elevation 860 m). Climate is Mediter-

ranean, with annual rainfall (average ca. 540 mm, with high inter–annual variability) concentrated mainly in spring and autumn, with very dry summers. Mean annual temperature is ca. 13°C. The landscape is characterised by moderate slopes, shallow acidic soils over a gneiss substratum and many rocky outcrops. Clearing of the original forest, where *Quercus ilex* subsp. *ballota* L. and *Juniperus oxycedrus* L. were the dominant tree species, resulted in a typical *dehesa* landscape with ca. 40 trees/ha over a grassland understorey with a high proportion of annual species.

Extensive grazing has been the dominant use in the region for centuries, but at the present time there is a great variability in grazing intensity within the area (Peco *et al.* 2005). In order to cover a wide range of situations, we selected sites in four different levels of grazing: *(1)* areas in which grazing has been abandoned; *(2)* areas grazed only occasionally; *(3)* areas where livestock is permanently present, but in which grazing pressure is moderate because they are distant from points of livestock concentration such as water and feeding points; *(4)* areas under permanent high levels of grazing because they are close to points of livestock concentration. We made sure that the grazing status of all the selected areas has remained unchanged for at least 30 years. Two different habitat types can be distinguished within each of the grazing levels: *(1)* Wet habitats, located at the bottom of slopes, characterized by their deep soils, and that receive water and nutrient inflows; *(2)* Dry habitats, on the upper slopes with shallower soils and nutrient and water outflows. The differing topographic position between the two types of habitats have led to differences in their levels of soil water availability, clay percentage, total nitrogen and soil organic matter (Peco *et al.* 2006).

In each of these eight *habitat x grazing* levels we selected 8–9 independent sites, always situated in areas with slopes under 5% (total of 66 sites). In order to corroborate the differences in soil water content between habitats, we collected and oven dried a soil sample (cylinder of 5 cm and 98.17 cm^3) from each site in April 2012. We defined the water content of these soil samples as the ratio of the mass of water and the dry weight of the sample. As expected, we found significant differences in the soil water contents of wet (33.04% $\pm$1.39; mean $\pm$SE) and dry habitats (14.60% $\pm$1.09).

Vegetation and functional traits sampling

In each site we situated three 20x20 cm sampling quadrats, always in the same relative positions (1 m N, E and W from the site centre). We

surveyed these quadrats twice, in the spring of 2009 (dry year, with a 50% of long–term average spring precipitation) and 2010 (wet year, with a 153% of average spring precipitation). We estimated the cover of each species in each quadrat using six cover classes: (0) absent; (1) cover $\leq$1%; (2) 1% <cover $\leq$12%; (3) 12% <cover $\leq$25%; (4) 25% <cover $\leq$50% and (5) cover >50%. Eventually, we assigned to each species the median value of its cover class.

We found a total of 177 species in the surveys. For each species we collected data on three functional traits – canopy height, specific leaf area (SLA) and seed mass – related to species strategies in response to disturbance and productivity. Canopy height (distance between the plant base and the highest photosynthetic leaf) was measured on 10 non–grazed mature individuals of each species, at least 25 m from each other, in the areas where the species was more abundant. Canopy height regulates plant responses to resource availability and disturbance and can be interpreted as an indicator of successional status and response to grazing (Westoby *et al.* 2002; Díaz *et al.* 2007a). SLA (mm^2/mg) was measured in the same individuals, dividing the leaf area by its oven–dried mass. SLA is an indicator of resource–use strategies, with low SLA values being associated with long–lived leaves, low relative growth rates and long residence times of nutrients (Westoby *et al.* 2002). Seed mass was obtained in most cases from Azcárate *et al.* (2002), and new measurements were taken for the species not included in that paper, following the same protocol (30 dry seeds per species). Large–seeded species produce large seedlings that survive better under difficult conditions such as shade, drought or defoliation, but have reduced colonization capacity due to the smaller number of seeds produced (Westoby 1998; Moles and Westoby 2004).

Functional diversity calculation

SLA, canopy height and seed mass values were log-transformed and standardized to a 0–1 scale. For each quadrat we calculated for each the community weighed mean values of each trait (CWM_{SLA}, CWM_{Height} and CWM_{Seed}):

$$CWM_j = \sum_{i=1}^{S} \frac{c_{ij} \text{ x } ti}{Cj} \tag{4.1}$$

where S is the species richness in the plot, c_{ij} is the cover of species i in plot j, $C_j = \sum c_{ij}$ and t_i is the average trait value (standardized and

transformed) for species i. CWM is generally understood as reflecting the average trait values of the dominant species in a community (Díaz *et al.* 2007b).

Afterwards, for each trait, we calculated a matrix of Euclidean distances between all possible species pairs. In order to have an indication of functional richness and functional divergence, we calculated the Rao quadratic entropy (henceforth *Rao*; Rao 1982; de Bello *et al.* 2010) for each trait in each quadrat and year. The Rao index can be expressed as:

$$FD_{Rao} = \sum_{i=1}^{S} \sum_{j=1}^{S} d_{ij} \times p_i \times p_j \tag{4.2}$$

where d_{ij} expresses the dissimilarity between each pair of coexisting species i and j and varies from 0 (two species with exactly the same trait values) and 1 (two species with completely different traits). p_i and p_j indicates the relative abundances of species i and j. We chose Rao because it has been found to be a reliable index when analysing changes in assembly processes across gradients of stress (Mason *et al.* 2012,in press) and can take species relative abundances into account (de Bello *et al.* 2010). For each quadrat and year we calculated two Rao values. The first one was calculated taking into account only species presences (i.e. assigning all species in a quadrat the same abundance value; Rao_{pr}). When calculated this way, Rao is an indicator of functional richness (Mason *et al.* 2012). For the second Rao value we took into account species abundances (Rao_{ab}). This is a combination of both functional richness and functional divergence.

Raw Rao values may be trivially influenced by species richness (i.e. may be correlated with species richness even in randomly generated data), especially when using presence/absence data. Consequently, relationships between Rao and environmental gradients can arise simply due to variation in species richness. To remove spurious effects of species richness on Rao values we compared observed Rao values with two different null models. First, we compared observed Rao_{pr} with null values generated using a matrix-swap null model (using 2000 initial swaps and 2000 swaps between calculation of each null value) based on the species presence-absence matrix (Manly 1995). Second, we compared observed Rao_{ab} with null values generated by randomizing abundances across species but within communities (Mason *et al.* 2008a; Hardy 2008). Comparing the observed Rao_{ab} values with the Rao_{ab} obtained in this

second type of randomization provides a pure estimation of functional divergence. Simulation studies indicate that these indices collectively provide a high level of power to detect changes in assembly processes along environmental gradients (Mason *et al.* in press).

We performed 10,000 randomizations of each null–model in order to calculate the standardized effect size (SESRao = [observed Rao – mean of simulated Rao]/standard deviation of simulated Rao; Gotelli and McCabe, 2002). For each site and year, we obtained two SES-Rao values by averaging the values of its three component quadrats: $SESRao_{pr}$ (observed (Rao_{pr} compared with the matrix swap null model) and $SESRao_{ab}$, (Observed Rao_{ab} compared with the null model randomizing abundances across species but within communities). For convenience we refer to them respectively as *FR* and *FD* henceforth.

In order to ascertain if grazing and habitat type affect the temporal variability of the studied traits, we calculated for each quadrat and trait the difference between its CWM, FR and FD values in the wet year and in the dry year. Positive values of these differences for a particular parameter indicate an increase in that parameter in the wet year compared with the dry one, and vice–versa. Interannual differences were calculated separately for each quadrat, then the differences for the three quadrats of each site were averaged, obtaining a single measurement of the interannual variability of CWM, FR and FD for each site.

Statistical analyses

We fitted repeated measures ANOVAs for each trait in order to examine the dependence of CWM, FR and FD on grazing level, habitat type and year, using year as the repeated measures factor. To ease interpretation of our results, we also fitted separate ANOVAs for each year; in these models, whenever we found a significant effect of the *grazing x habitat* interaction, we split the data and performed a Tukey–HSD post–hoc test on each habitat in order to detect differences between grazing levels. In the cases in which we found a significant effect of grazing, but not of the interaction, we looked for differences between grazing levels by performing a Tukey-HSD test without splitting the data.

The influence of grazing and habitat type on the indicators of inter–annual functional variability (differences between years in CWM, FR and FD) was also analysed by means of ANOVAs, following the same scheme explained above. All analyses were performed using R (R Development Core Team 2011).

4.3 RESULTS

Community weighted means

Grazing intensity had significant effects on the CWM values of all the traits studied and these effects were generally independent of habitat type (Table 4.1; Fig. 4.1). High levels of grazing increased CWM_{SLA} and decreased CWM_{Height}. The direction of the grazing effect was not altered by interannual changes in water availability. At the same time, the effect of habitat type on CWM was also consistent over time for SLA and canopy height; plants present on wet habitats were consistently taller and had lower SLA values than those in dry habitats (Fig. 4.1a). However, grazing and habitat type influenced temporal variability in both CWM_{SLA} and CWM_{Height}. SLA values tended to increase during the wet year in the high end of the grazing gradient and to decrease in the grazing abandoned areas (Fig. 4.2b). The interannual variability in CWM_{Height} in the dry habitats was strongly reduced by grazing (Fig. 4.2a).

Grazing tended to reduce CWM_{Seed}, but the post-hoc tests did not reveal any significant differences between grazing levels Grazing affected the temporal variability in CWM values of all the studied traits (Fig. 4.1c). The effect of habitat on CWM_{Seed}, varied between years. In the wet year seed size decreased markedly in the dry sites and increased markedly in the wet sites. Further, grazing modulated the temporal variability of CWM_{Seed}, with greater temporal fluctuations in the areas under the highest grazing pressures (Fig. 4.2c).

Functional richness and functional divergence

Neither FR_{SLA} nor FR_{Seed} were affected by grazing level in any of the studied scenarios. By contrast grazing significantly reduced FR_{Height} in dry habitats (Table 4.1a; Fig. 4.1). The relatively small effect of grazing contrasted with the very important influence of habitat type on FR. For vegetative traits, wet habitats had greater FR than expected by chance, whereas dry habitats generally had lower FR values than expected (Fig. 4.1a–b). On the contrary, the FR_{Seed} values were lower than expected in wet habitats and higher than expected in dry habitats, a tendency that became even more evident during the wet year (Figs. 4.1c and 4.2c).

In contrast with the lack of effects of grazing in the interannual variability in FR, habitat type played an important role in this aspect for FR_{Height} and FR_{Seed}, which increased during the wet year in dry habitats and remained relatively unchanged in wet habitats (Fig. 4.2). Neither grazing nor habitat type had a significant influence in the interan-

Table 4.1. Results of the repeated measures ANOVAs analysing the values of the community weighted mean (CWM), functional richness (FR) and functional divergence (FD) for each functional trait. Grazing level, habitat type and year as well as their interactions were used as explanatory variables. Year was the repeated measure. P values are shown in brackets. Significant results (P<0.05) are in bold.

	d.f.	SLA F	Height F	Seed mass F
Between factors			**a) CWM**	
Grazing level	3	**16.37 (<0.001)**	**9.28 (<0.001)**	**4.98 (0.004)**
Habitat	1	**123.19 (<0.001)**	**132.73 (<0.001)**	2.38 (0.128)
Grazing x Habitat	3	**3.36 (0.025)**	1.47 (0.232)	1.27 (0.293)
Residuals	58			
Within factors				
Year	1	**7.70 (0.007)**	0.08 (0.778)	**5.47 (0.023)**
Grazing x Year	3	**4.54 (0.006)**	**6.17 (0.001)**	0.03 (0.993)
Year x Habitat	1	**7.77 (0.007)**	0.61 (0.438)	**57.75 (<0.001)**
Grazing x Year x Habitat	3	0.24 (0.870)	**3.36 (0.025)**	**4.20 (0.009)**
Residuals	58			
Between factors			**b) Functional Richness**	
Grazing level	3	0.57 (0.639)	**7.28 (<0.001)**	1.02 (0.393)
Habitat	1	**122.75 (<0.001)**	**128.78 (<0.001)**	**99.11 (<0.001)**
Grazing x Habitat	3	0.23 (0.873)	**14.94 (<0.001)**	1.59 (0.201)
Residuals	58			
Within factors				
Year	1	0.93 (0.338)	3.36 (0.072)	0.04 (0.834)
Grazing x Year	3	0.72 (0.546)	1.82 (0.154)	0.270 (0.847)
Year x Habitat	1	0.20 (0.660)	**14.46 (<0.001)**	**16.16 (<0.001)**
Grazing x Year x Habitat	3	2.28 (0.089)	0.485 (0.694)	0.01 (0.999)
Residuals	58			
Between factors			**b) Functional Divergence**	
Grazing level	3	**5.21 (0.003)**	**3.43 (0.022)**	0.16 (0.921)
Habitat	1	**5.28 (0.025)**	**27.82 (<0.001)**	1.70 (0.197)
Grazing x Habitat	3	1.64 (0.191)	2.37 (0.080)	0.64 (0.595)
Residuals	58			
Within factors				
Year	1	**17.32 (<0.001)**	**6.36 (0.014)**	0.01 (0.922)
Grazing x Year	3	**3.24 (0.028)**	1.55 (0.212)	0.48 (0.698)
Year x Habitat	1	**49.70 (<0.001)**	0.19 (0.663)	0.12 (0.732)
Grazing x Year x Habitat	3	**5.21 (0.003)**	**5.26 (0.003)**	0.25 (0.863)
Residuals	58			

nual variability in FR_{SLA}, which remained rather constant among years (Fig. 4.2).

FD_{SLA} and FD_{Height} had lower values in dry than in wet habitats during the dry year (i.e. greater trait convergence between dominant species). Increased levels of grazing reduced the FD_{Height} values in dry habitats during the dry year, which were consistently lower than expected in the grazed areas (Fig. 4.1b). However, the effects of grazing and habitat type changed when productivity increased during the wet year: differences in FD_{Height} between habitats ceased to be significant, except in the most intensively grazed zones, in which wet habitats had higher than expected values of FD_{Height} (Figs. 4.1b and 4.2b). FD_{SLA} values in dry habitats experienced a generalized increase during the wet year, which reversed the relative positions of both habitats, resulting in significantly higher divergence in SLA values for dry than for wet habitats (Fig. 4.2a; Table 4.1b). None of the studied factors affected either the functional divergence of seed mass, which did not differ from random expectation, or its temporal variability (Table 4.1c).

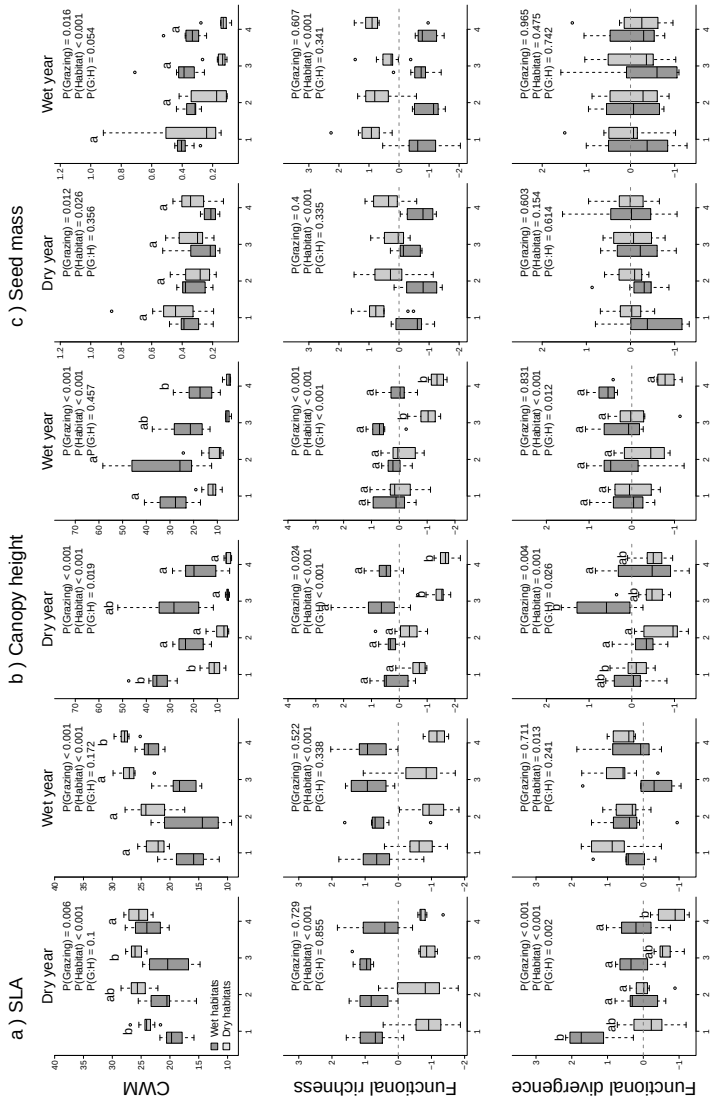

Figure 4.1. Boxplots showing the effect of grazing intensity and habitat type on the community weighted mean values (CWM; upper line), functional richness (medium line) and functional divergence (bottom line) of the different functional traits for each grazing level and habitat type during the dry (2009) and wet (2010) years. On each panel we show the p-values of grazing, habitat type and its interaction (G:H) obtained by means of ANOVA. When G:H is significant, different letters for each habitat type indicate significant differences between grazing levels (Tukey's test, $p<0.05$). When G:H is not significant, but grazing is, different letters indicate significant differences between grazing levels, pooling habitat types (Tukey's test, $p<0.05$).

4.4 DISCUSSION

Our results show that temporal variability in rainfall can have a great influence on the functional structure of plant communities – even between consecutive years. We found that temporal and spatial variation in resource availability interact with disturbance (i.e. herbivory) in complex ways to alter assembly processes. In addition, our results suggest that the assembly processes governing plant communities can vary markedly even over short timeframes. This in turn suggests that trait diversity may enhance biodiversity maintenance, since trait–mediated interspecific differences in response to temporal resource fluctuation may reduce differences in average fitness between co–occurring species (Chesson *et al.* 2000, 2004). Besides, the different traits and measures of functional structure that we used revealed contrasting patterns and showed varying power to detect the effects of resource availability and grazing. This result highlights the importance of the simultaneous use of complementary measures of FD based on individual traits rather than multiple combinations of traits.

Community weighted means

The CWM results evidence that the selected traits effectively captured species responses to temporal and spatial variation in resource availability and herbivore pressure. All the traits responded to grazing in a way consistent with literature, with higher SLA, shorter height and smaller seeds towards the higher end of the grazing gradient (McIntyre and Lavorel 2001; de Bello *et al.* 2005; Cingolani *et al.* 2005b, Peco *et al.* 2012; Laliberté 2012). Dry habitats had, on average, smaller species than wet ones, indicating increased importance of competition for light under higher resource availabilities and confirming that grazing and aridity select for similar trait values (Milchunas *et al.* 1988; Quiroga *et al.* 2010). In contrast, higher CWM_{SLA} values in dry than in wet habitats were against previous observations (Cornwell and Ackerly 2009; Douma *et al.* 2012), probably because of the higher abundance of annual species in the dry habitats, which have higher SLA values than perennials (Garnier 1992; Lavorel *et al.* 2011). The increase in CWM_{SLA} experienced in the wet year by the more intensively grazed areas in dry habitats (Fig. 4.2a) reveals interannual changes in the most successful resource–use strategies, which shifted from a resource–retaining strategy to a resource–acquiring strategy when water availability increased. This was caused by the remark-

able increase on the abundance of *Poa bulbosa*, a species with fast growth rates and a relatively high SLA value. This increase is probably related with the high sensitivity of *P. bulbosa* to water deficits (Mamolos *et al.* 2001) and with its increased grazing tolerance under high resource availability conditions (Staalduinen *et al.* 2010). Simultaneously, we observed decreases in the abundances of species with rosette form and intermediate SLA values such as *Leontodon taraxacoides* or *Hypochaeris glabra*, suggesting that grazing–avoidance strategies are favoured under dry conditions, while more humid conditions favour grazing–tolerance strategies (Grime 1977; Díaz et al 2007a). Interannual variability in CWM_{Seed} increased along with grazing intensity. However, the direction of this change depended on habitat type (Fig. 4.2c), resulting in smaller seeds in dry habitats during the wet year and bigger seeds in wet habitats. These results suggest that increased stress levels, manifested as increased shading in wet habitats during the wet year and as severe constrains in water availability in dry habitats during the dry year, select for large–seeded species with higher survivorship rates in stressed conditions (Moles and Westoby 2004).

Functional richness and functional divergence

Reduced water availability is predicted to increase convergence in traits related with light acquisition among the most abundant species, because there is no fitness advantage gained from niche differentiation when competition for below–ground resources prevails (Mason *et al.* 2012). This prediction was confirmed by the generalized increase in FD_{SLA} in dry habitats during the wet year. Stress alleviation in the wet year increased the diversity in SLA, probably because of an increased importance of competition for light. The increase of *Vulpia myuros*, a graminoid with a much lower SLA value than the dominant *P. bulbosa* contributed decisively to this increased divergence during the wet year (Fig. 4.2a). However, increased FD_{SLA} in dry habitats was not accompanied by any increase in FR_{SLA}, which remained rather stable among years and even decreased in the most intensively grazed areas. Thus, dry habitats in the wet year had simultaneously higher functional divergence and lower functional richness than null communities, a result that suggest that habitat filtering and limiting similarity are not mutually exclusive, but rather processes that take place hierarchically in natural communities (Cornwell and Ackerly 2009: de Bello *et al.* 2012). First, habitat filtering

reduces the viable pool of species by reducing the range of possible trait values (functional richness); then, limiting similarity causes the most abundant species to have different trait values (Mason *et al.* 2012). The

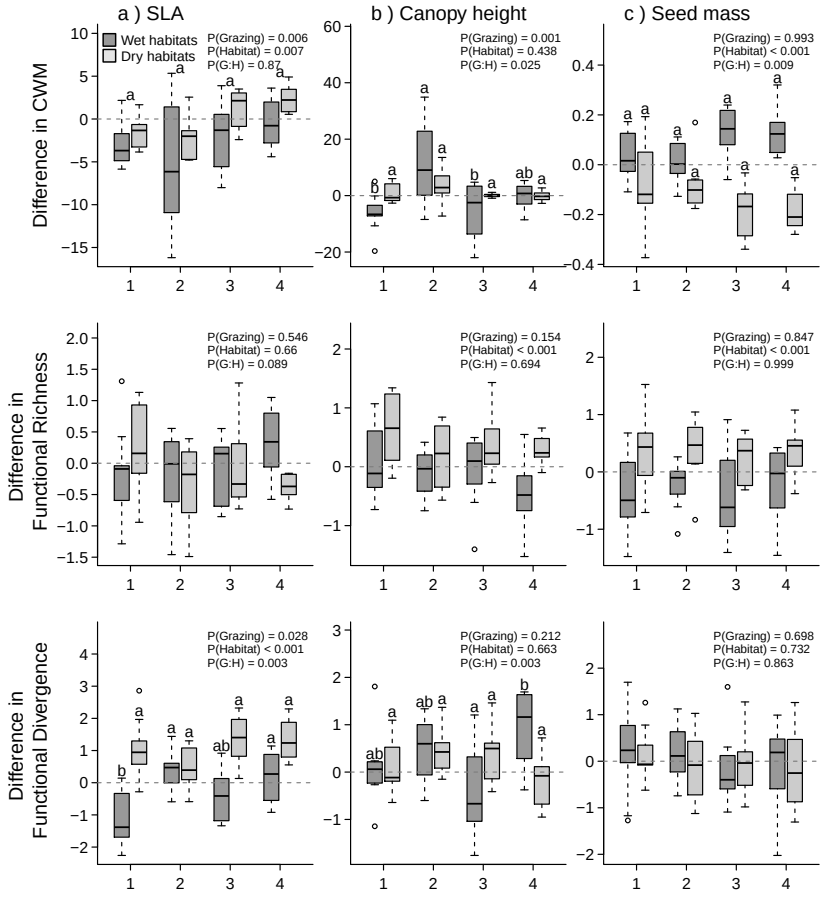

Figure 4.2. Boxplots showing the effect of grazing intensity and habitat type on interannual change in the community weighted mean values (CWM; upper line), functional richness (medium line) and functional divergence (bottom line) of the different functional traits for each grazing level and habitat type. The change was calculated by subtracting the value in the dry year (2009) from the value in the wet year (2010) for each site. Interpretation of the statistical results is as in Fig. 4.1.

interannual stability in FR_{SLA} (Fig. 4.2a), along with the marked differences among habitats (Fig. 4.2a), indicate that short–term (interannual) changes in the productivity level do not affect the range of SLA values, but that long–term and sustained differences, such as the ones produced by the different topographical positions between dry and wet habitats, can have a great influence on these values through changes in the pool of species.

The low values of FR_{Height} in dry habitats were further reduced by intense grazing, confirming that grazing reinforces the effects of aridity on vegetative functional traits. FR_{Height} increased more in dry than in wet habitats, because of the appearance of relatively tall species in dry habitats, such as *Xolantha guttata* or *Vulpia myuros*, which increased the range of height values in these sites. The presence and abundance of these species is known to be greatly influenced by yearly fluctuations in rainfall (Peco *et al.* 1998), as confirmed in this study. Increased values of FD_{Height} during the wet year, especially in the most intensively grazed areas, suggest an increase in the rate of biomass replacement due to higher resource availability. The short growing season during the dry year resulted in a reduction of the capacity of plants to compensate for the biomass removed by grazing, especially for tall species that need long growing seasons (e.g. *Agrostis castellana*, *Festuca rothmalieri*). However increased productivity during the wet year allowed these species to replace biomass lost to herbivory, which finally resulted in an increase in the abundance of tall species in the more intensively grazed areas. Higher resource availability increased the relative importance of the competition for light, resulting in an stronger role of limiting similarity, with the subsequent reduction in the abundance of species with intermediate height values (e.g. *Juncus articulatus*, *Isolepis setacea*, *Mentha pulegium*), along with an increase in the abundance of short species (e.g. *Poa annua*, *Trifolium subterraneum*). Thus, increased productivity generated greater functional divergence by enhancing the abundances of species situated towards the extremes of the trait range, and reducing the abundance of species close to the centre of this range, a result that could not have been observed by merely inspecting the change in CWM values.

The FR_{Seed} in dry sites increased during the wet year, associated with a reduction of the average seed mass values in these sites. This was caused by the increased presence and abundance of small–seeded species such as *Xolantha guttata*, *Spergula arvensis* or *Vulpia myuros*, along

with the reduction of species with heavy seeds during the wet year (e.g. *Astragalus pelecinus*, *Brassica barrelieri* or *Erodium cicutarium*). This interannual change was likely to be caused by the bigger capacity of seedlings of large–seeded species to survive under stress conditions, such as drought (Leishman *et al.* 2000b; Moles and Westoby 2004). At the same time, small seeds are known to have longer persistence in the seed bank than large ones (Thompson *et al.* 1993; Traba *et al.* 2003). Thus, enhanced germination and seedling survivorship of the small–seeded species present in the seed bank, would explain the great increase in the number of these species during the wet year.

Concluding remarks

In the studied systems, aridity and grazing reinforced their effects in a hierarchical way, generally reducing the range and divergence of the vegetative trait values that can be found in a site and hence its functional diversity. In agreement with our second hypothesis, functional richness values for vegetative traits were higher in wet habitats than in dry ones. Besides, these differences were reduced during the dry year, as we expected (hypothesis 3). Therefore our results confirm that, under increased competition for light, niche differentiation in vegetative traits is necessary for species coexistence. In contrast, functional richness for seed mass was remarkably higher in dry than in wet environments, revealing the simultaneous existence of trait convergence and divergence for different niche axes. This result remarks the importance of analysing individual traits rather than just using multivariate trait indices (Spasojevic and Suding 2011). Moreover, the different indicators we have used to quantify trait convergence and divergence were often decoupled, underscoring the convenience of using different diversity indices simultaneously when assessing the effects of environmental variables on assembly processes (Ricotta and Moretti 2011; Mason *et al.* 2012).

The hypothesized reduction in the interannual variability of the studied indicators in the highest end of the grazing gradient was not observed, with the only exception of CWM_{Height}. These observations support the idea that in semi–arid ecosystems, water availability plays a major role in the determination of plant fitness, while the relative importance of disturbance is reduced (Kikvidze *et al.* 2011). Nevertheless, grazing influenced the average values of the vegetative traits (Height and SLA) and increased the convergence in canopy height values of the

species in dry habitats, confirming its role as a determinant of the diversity of grasslands (Carmona *et al.* 2012).

Altogether, our results show that, in plant communities dominated by annual species, interannual fluctuations in resource availability have a great influence in the assembly processes, through the promotion of the diversity of trait values, playing an important role in the maintenance of diversity (Chesson *et al.* 2004). Habitat type, closely related to productivity, and grazing intensity were confirmed as important determinants of the importance and direction of this temporal change.

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Chapter 5

Taxonomical and functional diversity turnover in Mediterranean grasslands: interactions between grazing, habitat type and rainfal

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Abstract

Changes in livestock grazing regimes are among the most important drivers of species loss and decrease of functional diversity worldwide. However, taxonomic and functional diversities (TD and FD) can respond differently to changes in grazing regime or productivity.

We surveyed plant communities from 67 sites under different grazing regimes (from heavy grazing to grazing abandonment) in wet and dry habitats, in both wet and dry years. We tested the influence of grazing intensity, habitat type and rainfall on TD, FD and the relationship between them. We also partitioned diversity to examine the effects of grazing on TD and FD across scales (within communities, within grazing levels and between grazing levels).

The effect of grazing within and across communities was modulated by water availability, with grazing showing the strongest effects in dry habitats. The relationship between FD and TD varied between habitat types and years and revealed high functional similarity between species (i.e. redundancy) in dry habitats. TD was reduced in the driest conditions across all the observation levels, contrasting with the high temporal stability of FD, suggesting that FD was decoupled from TD, especially in dry habitats. However, despite the high temporal and spatial stability of FD, results show that under severely limited water availability, high grazing pressure can reduce FD, revealing a convergence in traits under the combined effect of grazing and drought conditions.

Synthesis and applications. Results highlight the dependence of functional diversity on the combined effect of water availability and grazing regime. Under severely limited water availability, grazing intensification reduced the functional diversity of these grasslands. Because of the foreseeable reduction in water availability in Mediterranean environments, we recommend the adoption of flexible grazing management schemes that take species and functional diversities into account simultaneously and adapt the level of grazing pressure to water availability.

5.1 INTRODUCTION

Land use change is one of the most important drivers of biodiversity loss and ecosystem services changes worldwide (MEA 2005). In many areas of the world, changes in livestock management are leading to profound changes in plant biodiversity patterns (Bakker *et al.* 2006). Grazing and water stress are often considered convergent selective forces selecting for similar plant traits (Milchunas *et al.* 1988; Quiroga *et al.* 2010). Consequently, it is generally expected that changes in grazing management would have a more pronounced effect on biodiversity in wetter areas (Cingolani *et al.* 2005a).

Although taxonomic diversity (TD) has received most attention in the literature, biodiversity includes multiple components, beyond the number of species. Among them, functional diversity (FD, i.e. the extent of trait differences between species in a community; Díaz and Cabido 2001) can reveal why biodiversity changes in response to environmental change, as well as how biodiversity influences ecosystem functioning (Díaz and Cabido 2001). As a result, there is increasing interest in the study of FD and its dependence on environmental conditions and management (de Bello *et al.* 2006; Mayfield *et al.* 2010).

European pastures, particularly in Mediterranean environments (Sluiter and Jong 2006), are under a dual process of abandonment in the less productive areas and intensification in the more productive or accessible ones (Stoate *et al.* 2009). Grazing abandonment affects floristic composition, functional traits such as canopy height, leaf dry weight, onset of flowering, life form and seed mass (Peco *et al.* 2005; Díaz *et al.* 2007a; Peco *et al.* 2012), the overall FD of communities (de Bello *et al.* 2006) and many environmental aspects such as light quality and intensity and soil characteristics (Peco *et al.* 2006). In contrast, intensification processes have been found to reduce FD, although these results depend on the studied site or taxonomic group (Flynn *et al.* 2009). Plant diversity in the Mediterranean areas of Europe is expected to be greatly impacted because of the predicted reduction in rainfall (Thuiller *et al.* 2005). Nevertheless, relatively few studies have related the response by FD to changes in land use (e.g. Flynn *et al.* 2009; Mayfield *et al.* 2010) or climate (Thullier *et al.* 2006), and even fewer have assessed how the functional turnover within and between management types is affected by environmental or management conditions (de Bello *et al.* 2009). In particular, it is uncertain whether the changes in TD will be mirrored by changes in FD within and among communities.

There are a number of reasons to suspect that TD and FD do not always covary in response to land-use changes (Mayfield *et al.* 2010), thus characterizing their relationship provides insights into the consequences of different disturbance regimes or management strategies on community assembly and ecosystem functioning (Sasaki 2009b). For example, assemblages that have functional redundancy, contain species that have similar functional traits, and thus the addition of these species does not increase FD (Petchey *et al.* 2007). On the contrary, the extinction of a functionally unique species can compromise the stability of a given community (Micheli and Halpern 2005). Thus, functional redundancy can be examined through the relationship between TD and FD - namely if TD increases and FD remains constant. Factors such as productivity (Sasaki 2009b) or disturbance regime (Biswas and Mallik 2011) modulate this relationship because they can select for, or against, convergent types of traits (Grime 2006). For example, grazing can increase or reduce grassland diversity, depending on productivity, grazing history and intensity (de Bello *et al.* 2006; Bakker *et al.* 2006).

These factors affect diversity within but also between communities (Milchunas *et al.* 1988). Consequently, to understand its effects across habitats, it is necessary to partition the total diversity of the studied system (γ) into its α (within communities) and β (among communities) components (Whittaker 1972), and examine their temporal variability. Partitioning diversity also allows us to understand how different community assembly rules act simultaneously at different scales (de Bello *et al.* 2009). In this paper we study how environmental gradients affect community structure and examine how the mechanisms that control coexistence in plant communities are regulated by the productivity and disturbance levels. We also want to provide an insight into the potential effects of land-use change, combined with the foreseeable reduction in water availability, on Mediterranean grasslands at a landscape level. In particular, we focus on how habitats with different levels of productivity, linked mainly to differences in water availability, affect both TD and FD across a gradient of grazing pressure. We also study how these parameters are affected by inter-annual rainfall variability by comparing two years that differed notably in the amount of precipitation. Finally, we examine whether, and to what extent, water availability determines the spatial partitioning of both TD and FD in a landscape under several grazing intensities. Specifically, our hypotheses were: *(1)* due to a stronger effect of habitat filtering, FD should be lower in dry than in wet

conditions, *(2)* since grazing and aridity select for similar traits, differences in diversity among different levels of grazing should be higher in wet conditions than in dry ones, *(3)* γ diversity, as well as the proportion of diversity explained by the variability between grazing levels, should be higher in wet than in dry conditions.

5.2 MATERIAL AND METHODS

Study area

The study area (5 x 4 km) is 35 km North of Madrid, Spain (40°38′ N; 3 °70′ W; mean elevation 860 m). Climate is Mediterranean, with dry summers and maximum precipitation in spring and autumn. Annual rainfall is 540 mm, with large interannual fluctuations, and mean temperature is 13 °C. The landscape is characterised by gentle slopes (<5%), shallow acidic soils over a gneiss substratum and many rocky outcrops. The area is covered by dehesas, open woodlands (ca. 40 trees ha^{-1}) of *Quercus ilex* subsp. *ballota* L. and *Juniperus oxycedrus* L. , with a grassland understorey with a high proportion (ca. 70%) of annual species.

The area has been grazed extensively for centuries, but recent abandonment of grazing has created a mosaic of grazing pressure (Peco *et al.* 2005). We selected sites in four categories of increasing grazing intensity: (1) areas abandoned by ranchers and no longer grazed, (2) areas only grazed occasionally, with no permanent grazing, (3) areas with a permanent presence of livestock but away from points of livestock concentration (water points and points where animals are provided with feed), and (4) areas around points of livestock concentration, with permanent high levels of grazing. All the selected areas have maintained their current grazing status for at least 30 years. Within each of these levels, we identified two different habitat types: (1) wet habitats, at the bottom of slopes and depressions, with deep soils, water and nutrient inflows; and (2) dry habitats along the upper slopes, with shallow soils and nutrient and water outflows. Previous studies in the same area found differences in soil water availability, clay percentage, total nitrogen and soil organic matter between these two habitat types (Peco *et al.* 2006). We selected 8-9 independent sites in each of the eight grazing by habitat levels for a total of 67 sites. The average distance between adjacent sites was 106 m (minimum of 45 m), and the slope was always under 5%. To estimate differences between soil water content between habitat types, in

April 2012 a soil sample (cylinder of 5 cm and 98.17 cm^3) from each site was collected and oven-dried. Its water content was defined as the ratio of the mass of water and the dry weight of the sample. As expected, there were great differences in soil water content between wet (33.04% $\pm$1.39; mean $\pm$SE) and dry habitats (14.60% $\pm$1.09).

Vegetation and functional traits sampling

In each site, we placed three sampling quadrats (20x20 cm), always in the same relative positions (1 m N, E and W from the site centre). Species cover was estimated using six classes: (0) absent; (1) cover <1%; (2) 1–12%; (3) 12–25%; (4) 25–50% and (5) >50%. After assigning to the species the median value of its cover class, we averaged their cover across the three quadrats in each site. Vegetation was monitored in exactly the same quadrats in the springs of a dry (2009, 50% of average spring precipitation) and a wet year (2010, 153%). A total of 177 species were found in the surveys.

We collected data for eight functional traits related to species strategies in response to disturbance and productivity for a sufficient number of species (Pakeman and Quested 2007). Canopy height (distance between the plant base and the highest photosynthetic leaf) was measured on 10 non-grazed mature individuals of each species, at least 25 m from each other, in the areas where the species was more abundant. Specific Leaf Area (SLA; $mm^2\ mg^{-1}$) was measured in the same individuals, dividing the leaf area by its oven-dried mass. Seed Mass and presence of dispersal structures was obtained in most cases from Azcárate *et al.* (2002), and new measurements were taken for the species not included in that paper, following the same protocol (30 dry seeds per species). Information on the remaining traits (Life form, Growth form, Longevity, and Clonality) was taken from literature (Valdés *et al.* 1987).

Taxonomical and Functional diversities calculation

Quantitative traits were log-transformed and standardized to a 0-1 scale. For each trait, we calculated a matrix of distances between all possible species pairs and then tested the correlation between traits by performing Mantel tests between their dissimilarity matrices (not shown). For FD calculations we selected four traits with a very low level of correlation (Canopy height, SLA, Seed mass and Clonality). These traits have

been described as indicators of ecosystem functions, plant dispersal, establishment, persistence and response to grazing (Weiher *et al.* 1999; Díaz *et al.* 2007a), and represent an extension of the LHS scheme (leaf, height, seed traits; Westoby 1998) with the inclusion of clonal trait information (Klimešová and de Bello 2009). With these traits, we computed a new matrix of species functional dissimilarities using Gower distance and used it to calculate FD.

We calculated TD and FD at different spatial scales in order to have a comparable spatial partitioning of diversity for both of them. FD for each sampling site was calculated on the basis of the Rao index of diversity (Lepš *et al.* 2006):

$$FD_{Rao} = \sum_{i=1}^{S} \sum_{j=1}^{S} d_{ij} \text{ x } p_i \text{ x } p_j \tag{5.1}$$

The parameter d_{ij} expresses the dissimilarity between each pair of coexisting species i and j and varies from 0 (two species with exactly the same traits) and 1 (two species with completely different traits). In the case of binary or categorical traits, when the species has the same trait value, then $d_{ij} = 0$; otherwise, $d_{ij} = 1$. FD_{Rao} is a generalization of the Simpson index of diversity (Lepš *et al.* 2006), i.e. when all distances between species are equal to 1, FD_{Rao} is the Simpson index of diversity (i.e. 1-Simpson dominance, which was used to characterize TD). FD_{Rao} is among the few indices, if not the only one, that allows a comparable diversity partitioning of taxonomical and functional diversities with the same mathematical formulation, while taking species relative abundances into account (de Bello *et al.* 2010).

We computed α- (within sampling sites; expressed in equation 1), β- (between sites) and γ-diversity (within habitat type and grazing intensity) for both TD and FD. To calculate γ-diversity we pooled samples of habitat type, grazing intensity and year. β-diversity for both TD and FD was specifically expressed in percentage over γ, as β=(γ-meanα)*100/γ (details in de Bello *et al.* 2010). According to this formulation, β-diversity summarizes the proportion of between-sites diversity with respect to the total diversity within a grazing intensity zone, habitat type and year. In another analysis, we added a new level and partitioned diversity by considering the diversity between grazing intensity zones (within year and habitat type). We thus had 3 levels for each year and habitat type: within sites, between sites (within grazing levels) and between grazing intensity

levels. To calculate the abundance of species at different spatial scales, we applied the methods described in Villeger and Mouillot (2008) and de Bello *et al.* (2010). Finally, using the Rao index, we also calculated β-diversity between all possible pairs of the 67 sampling sites, for both TD and FD using the R function Rao (de Bello *et al.* 2010). This resulted in two dissimilarity matrices expressing the taxonomical and functional turnover between all possible pairs of sites To partition α and β diversity correctly, we applied a correction used in the context of the Rao index (de Bello *et al.* 2010), which involves first calculating the diversity for single sites (α) according to equation 1. For TD, α is expressed as 1 over dominance (where dominance is computed by $\sum_{i=1}^{S} p_i^2$) and for FD, it is equal to $1/(1\text{-}FD_{Rao})$.

Statistical analyses

We fitted repeated measures ANOVAs (type III sum of squares) to examine the relationships of α-TD and α-FD with grazing level (categorical with four levels), habitat type and year, using year as the repeated measures factor. Whenever we found significant values for the interactions between the variables, we fitted separated models for each habitat type and year, using only grazing level as explanatory variable, followed by Tukey-HSD tests in the case of a significant effect of grazing.

We then checked whether grazing, habitat and year influenced the dependence of α-FD on α-TD. We fitted a repeated measures ANCOVA in which α-FD of the sites was used as response variable and the explanatory variables were α-TD, α-TD^2 (to account for non-linear relationships), grazing level, year (repeated measures factor), and habitat type, as well as the interactions of α-TD with year and habitat type. In the case of a significant effect of the interactions, a different model for each year and habitat type was fitted using α-TD, α-TD^2, grazing level, and the interaction α-TD x grazing level. These models were simplified by selecting significant variables through backward stepwise regressions.

We then analysed the effect of grazing and habitat type on the taxonomical and functional turnover (β) between sites. We calculated the taxonomical and functional dissimilarities between each pairs of sites using the Rao index and analysed the resulting dissimilarity matrix with PERMANOVA (R package vegan; Oksanen *et al.* 2011) in which habitat type, grazing intensity and their interaction were used as explanatory variables. To visualize these analyses we performed a Non-metric

Multidimensional Scaling (NMDS) for each year based on the pairwise sites dissimilarities for both TD and FD. The same distance matrices were used to calculate the distance of each site to the centre of its class centroid (PERMDIST; Oksanen *et al.* 2011) and calculate the homogeneity of sites within each grazing intensity level and habitat type and year. We then analysed the PERMDIST results with repeated measures ANOVAs as for α-TD and α-FD (for each site we considered as response variable its distance to the centroid within each grazing intensity level, habitat type and year). Finally, for each year and habitat type we calculated and plotted the partitioning of TD and FD, as explained in the previous section. All analyses were performed using R (R Development Core Team 2011).

5.3 RESULTS

The response of α-TD and α-FD to grazing was different between the different habitat types (significant grazing x habitat type interaction; Table 5.1a). For α-TD there was a stronger response to grazing in the more humid conditions (wet habitats, wet year; i.e. the ANOVAs for each habitat type and year revealed significant differences between levels of grazing in wet conditions; Fig. 5.1a). In these conditions, α-TD increased with higher grazing pressure (Fig. 5.1a). Conversely, grazing affected α-FD negatively in the driest conditions (i.e. dry habitats, dry years), but it had no effect in the other scenarios. Interannual variability of α-TD was much higher in dry habitats, where α-TD exhibited a pronounced increase in the wet year compared to the dry one. In contrast, α-FD remained rather stable between years (Table 5.1a; Fig. 5.1a).

The relationship of α-FD with α-TD at the site level depended strongly on habitat type (α-TD x habitat type interaction P=0.004) and year (α-TD x year interaction P=0.005), and thus we fitted models for each combination of habitat type and year. Neither grazing nor the interaction α-TD x grazing were significant in any of the scenarios, and were consequently excluded from the final models. During the dry year, the selected models revealed a non-linear relationship between α-TD and α-FD indicating that beyond a given threshold, the addition of new species did not increase FD at the site level, but rather increased the functional similarity between species, especially in the dry habitat (Fig. 5.2a). In the wet year, the relationship between α-FD and α-TD was linear, either positive in the wet habitats and negative in the dry habitats (Fig. 5.2b).

The NMDS and PERMANOVA analyses showed that habitat type was the main factor controlling both taxonomical and functional turnover between sites (Fig. 5.3). Regarding the taxonomical dissimilarity, we observed an important differentiation between dry and wet habitats in the two years, and a remarkable increase in the differences between sites in the dry habitats during the wet year compared to the dry one, when these sites were much more alike. This implies that differences between dry and wet habitats are reduced in a wet year. The effect of grazing was small compared to that of habitat type, and differed between wet and dry habitats in both years, especially during the dry year. The significant interaction between habitat type and grazing observed for TD was also detected for FD in the dry year, when the sites of the wet and dry habitats were functionally more similar for lower than for higher levels of grazing. In contrast, during the wet year, the functional composition of both habitat types was similarly affected by grazing. Increased grazing also increased functional homogeneity, for both wet and dry habitats, but did not affect the taxonomical β diversity of wet sites (Table 5.1b). In contrast, the effect of grazing during the wet year was less important, and we only found a small, non-linear effect of grazing on the β-TD of dry habitats (Fig 1b).

The overall partition of TD revealed a remarkably higher total (γ) diversity in the dry habitat during the wet year (11.95) compared to the

Table 5.1. Results of the repeated measures ANOVAs. Grazing level, habitat type and year as well as their interactions were used as explanatory variables and year as the repeated measure. α and β Taxonomical and Functional diversities were the response variables. Significant results ($P<0.05$) are in bold.

	d.f.	α-TD F	α-FD F	β-TD F	β-FD F
Between factors					
Grazing level	3	**2.80**	0.55	**2.82**	**10.33**
Year	1	0.75	0.15	0.51	0.84
Habitat	1	0.15	**15.43**	**17.83**	**26.22**
Grazing x Habitat	3	**4.50**	**5.06**	**3.04**	2.41
Residuals	58				
Within factors					
Year	1	**46.22**	0.98	**25.57**	**4.15**
Grazing x Year	3	0.17	1.02	1.04	**4.48**
Year x Habitat	1	**45.43**	1.42	**19.54**	0.70
Grazing x Year x Habitat	3	2.43	1.73	**2.83**	0.29
Residuals	58				

dry year (3.56; Fig. 5.4). TD was more evenly distributed between observation levels in wet than in dry habitats, where most of the taxonomical variability could be observed at the site level. Nevertheless, there was a shift in the proportion of the variability that was observed at each level in dry habitats: while in the dry year most of the TD in the dry habitats was observed within sites (78.7% over the total diversity within habitat and year), this proportion was smaller during the wet year (60.7%), mainly because of the increase of the variability within grazing zones (12.5% in the dry year, 28.6% in the wet one). In contrast, most of the observed

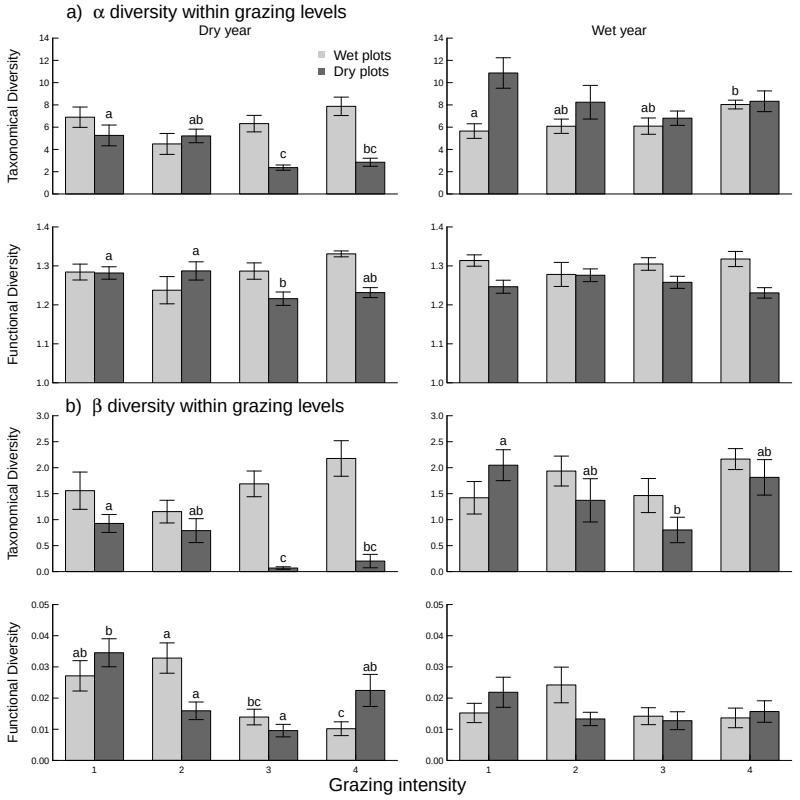

Figure 5.1. Response to grazing pressure of α and β Taxonomical and Functional diversities for each year and habitat type. Error bars show mean $\pm$ standard error. Different letters indicate significant differences between grazing levels (Tukey's test, $P<0.05$).

variability in FD (around 93% for dry habitats and 88% for wet habitats) took place at the within-site scale, regardless of habitat type and year. The proportions of FD explained by each level were much more independent of conditions (habitat and year) than those of TD. In this way, it is remarkable that the huge increase in γ-TD in the dry habitat during the wet year was not accompanied by any increase of γ FD in these areas, but rather a small decrease (1.66 vs 1.61; Fig. 5.4).

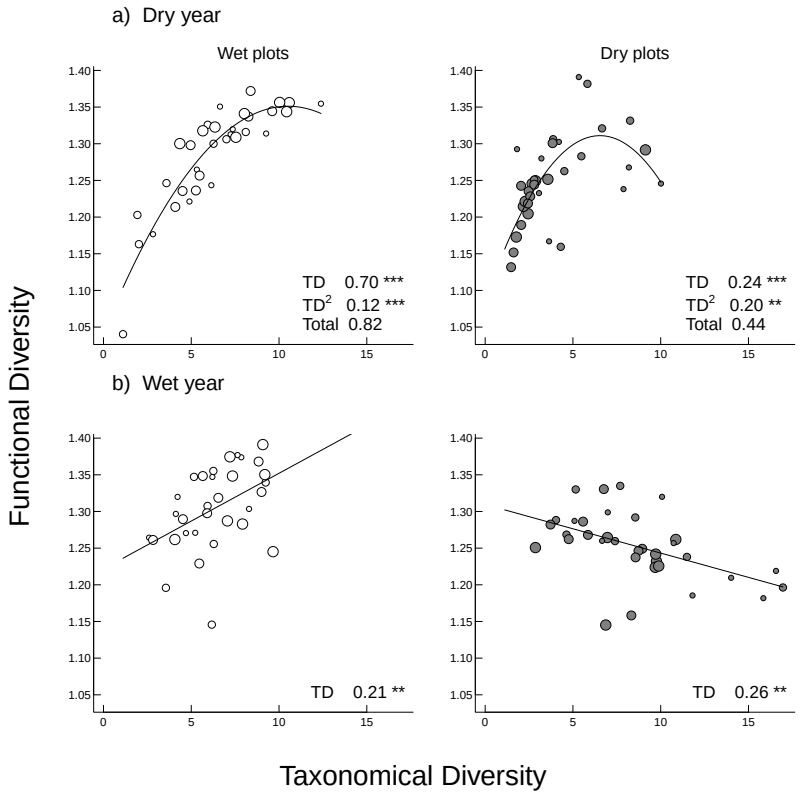

Figure 5.2. Relationship between α Functional and Taxonomical diversities. In each scenario, the most parsimonious model for each year and humidity level is represented. Point size reflects grazing intensity (bigger points indicate higher grazing levels). Each plot shows the proportion of variance in FD explained by each of the explanatory variables (TD: Taxonomical diversity; TD^2: squared Taxonomical diversity) and its significance level (*:$P<0.05$; **:$P<0.01$; ***:$P<0.001$).

5.4 DISCUSSION

Our main objective was to examine the combined effect of grazing and water availability on the diversity of Mediterranean grasslands, along with the processes that determine these effects. Water availability was the major determinant of both taxonomical and functional compositions, confirming results reported in many other studies (e.g. Cingolani *et al.* 2003; Sasaki *et al.* 2009a). In these semi-arid ecosystems, plants fitness is strongly affected by reduced water availability, making the relative importance of disturbance less evident (Kikvidze *et al.* 2011). However, the effect of grazing was significant in all the analysed scenarios, corroborating the importance of grazing in the diversity and composition of these systems at multiple spatial scales (Peco *et al.* 2005; Golodets *et al.* 2011).

Water availability also modulated the effect of grazing on TD and FD as well as the relationship between them. Thus, our results suggest that the effect of grazing on various diversity components was contingent on water availability. Increased water stress (dry year in dry habitats) resulted into a negative effect of grazing on α-TD, while this effect was positive when water availability was high (wet year in wet habitats), confirming that the response of α-TD to grazing varies along a gradient of water availability (Milchunas *et al.* 1988; de Bello *et al.* 2005; Bakker *et al.* 2006). This observation supports the idea that in more productive conditions, grazing reduces the relative importance of competitively dominant species and promotes species diversity through an increase in subordinate species, while in less productive conditions, grazing reduces species diversity by increasing plant mortality, e.g. excluding the most palatable species (Bakker and Olff 2003). This is consistent with predictions for areas with a long history of grazing (Milchunas *et al.* 1988; Cingolani *et al.* 2005a) and shows that not only regional but also local differences in productivity within a climatic region can affect the relationship between TD and grazing (Sasaki *et al.* 2009b). Local differences in productivity can be particularly important in semi-arid systems like oak savannas, because small changes in productivity can change the direction of the effect of grazing on diversity (Bakker *et al.* 2006). However, our results contrast with previous studies in the same area, which found no differences in the species richness between grazed and ungrazed conditions (Peco *et al.* 2006,2012). The inclusion of more grazing levels in the present paper, especially high grazing pressure, may have caused these differing results. Climatic conditions affect not only species composition

of these communities, but also modify the effect of grazing on TD, as shown in this study. Consequently, studies analysing the effects of grazing on plant diversity should take into account interannual differences in water availability.

Contrary to our expectations, and to the response of α-TD, the effect of grazing on α-FD was greater in dry than in wet conditions. Grazing

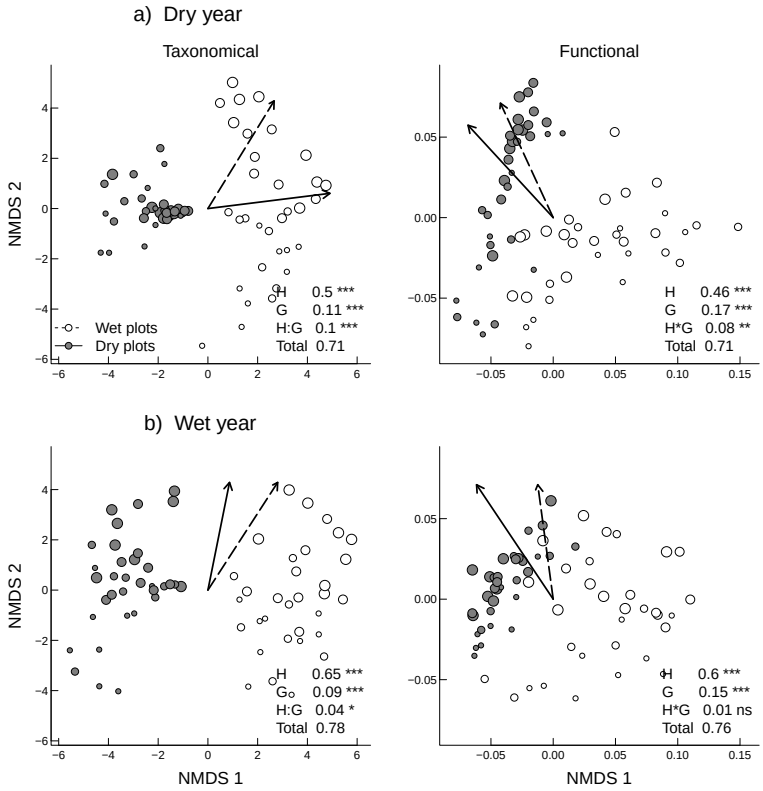

Figure 5.3. Results of the Non-metric multidimensional scaling, showing the effects of grazing and humidity on the Taxonomical and Functional compositions in dry and wet years. Arrows show the effect of grazing for dry sites (continuous line) and wet sites (discontinuous line). Point size reflects grazing intensity (bigger points indicate higher grazing levels). Each plot shows the results of the PERMANOVA analysis (9999 permutations): proportion of explained variance by the explanatory variables (H: Habitat; G: Grazing; H*G: Habitat x Grazing) and its significance level (*:P$<$0.05; **:P$<$0.01; ***:P$<$0.001).

reduced α-FD under conditions of increased water stress (dry habitats in dry year), contrasting with its lack of effect in wetter conditions (Fig. 5.1). Although grazing and aridity are often considered convergent selective forces (Milchunas *et al.* 1988; de Bello *et al.* 2006), this pattern is possibly not universal (e.g. plant height in Quiroga *et al.* 2010). Rather than supporting the role of disturbance as the principal factor creating and sustaining divergence in trait values (Grime 2006), our results agree with those of Pakeman *et al.* (2011), considering disturbance (grazing in this case) as a filter that can cause trait convergence within plant communities and suggest that its strength as a filter for trait values depends on productivity. In particular, the combined effect of stress and disturbance might reduce the functional strategies available in a site.

Grazing intensity effects, while different for α-FD and α-TD, did not affect the slopes of the relationship between them in any of the studied cases, in contrast with the findings of Biswas and Mallik (2011). Contrary to the conclusions of studies that evaluated this relationship as positive and linear, regardless of environmental conditions (Micheli and Halpern 2005, Sasaki *et al.* 2009b), we found that both the shape and the strength of the relationship depended on water availability. While this relationship was positive and linear over most of its range in wet habitats, in dry habitats it was negative and linear during the wet year and hump-shaped during the dry year. Our results highlight the importance of considering both FD and TD in the assessment of ecosystem responses to disturbance and stability (Mayfield *et al.* 2010). Nevertheless, care should be taken when comparing our results with other studies, because different FD indices can be influenced differently by species richness (Poos *et al.* 2009) and can have different ecological interpretations (Mouchet *et al.* 2010). Such comparisons are currently also limited by the small number of studies that consider both TD and FD (Cadotte *et al.* 2011).

Negative and hump-shaped relationships between α-FD and α-TD have been previously reported in animal communities (e.g. Mason *et al.* 2008b) and plant communities after changes in land use (Mayfield *et al.* 2010). Above a determined level of α-TD, we observed increasing levels of redundancy, i.e. similarity in functional traits of the species that result in a weaker or non-positive, relationship between α-FD and α-TD (Petchey *et al.* 2007). Below that level, reduced redundancy of the communities (Petchey *et al.* 2007), would explain the abrupt reduction in α-FD. This was especially evident in the driest conditions, in which communities under high grazing pressure presented a very strong relation-

ship between α-FD and α-TD (Fig. 5.2a), resulting from the effect of grazing on the more palatable species (Díaz *et al.* 2007a, Sasaki *et al.* 2009a). The non-linear relationship was only observed during the dry year because great increases in α-TD in dry sites during the wet year masked the segment of the relationship dominated by reduced redundancy. This functional convergence is consistent with the notion of habitat filtering (Cornwell and Ackerly 2009; Pakeman *et al.* 2011), indicating that only a subset of the possible trait values were found in the harshest conditions. Dry and wet habitats differed in the levels of α-TD at which α-FD peaked (higher in wet habitats), indicating higher functional redundancy in dry habitats due to a stronger role of habitat filtering. Nevertheless, we are aware of the potential confounding effects that the existence of a legacy effect of drought on the growth of perennial species could have on our results (de Vries *et al.* 2012).

Partition of diversity revealed important differences between TD and FD. As expected, the proportion of the total (γ) TD due to differences between grazing levels was remarkably higher in wet than in dry habitats. This was especially evident during the dry year, when the harsh conditions imposed a great reduction in γ-TD in the dry habitats in comparison with the wet ones and minimized the proportion of γ-TD due to differences between grazing levels and between sites at the same grazing level (Fig. 5.4). Small differences between grazing levels in these conditions indicated that aridity and grazing operate as convergent forces (Quiroga *et al.* 2010). However, grazing reduced taxonomical differences between sites within the same grazing level (Fig. 5.1b), which contradicts the former observation. This suggests that among the small number of species that are able to survive under severe water restrictions, most but not all of them can cope with high grazing pressures (Cingolani *et al.* 2005a). On the other hand, both γ-FD and its spatial partition were less dependent on climatic conditions and did not vary among years, suggesting that FD is largely independent of TD in dry habitats because of the high functional redundancy. As expected, the proportion of FD explained by within-site variability was smaller in wet than in dry habitats, but this difference was smaller than for TD. Most functional variability was found to be at the site level, which is consistent with previous studies (Cornwell and Ackerly 2009; de Bello *et al.* 2009) and with the hypothesis of limiting similarity as a mechanism promoting trait divergence within communities (Wilson 2007). Our results support the idea that habitat filtering and limiting similarity are not mutually exclusive,

but mechanisms that can take place hierarchically in a community (de Bello *et al.* 2012).

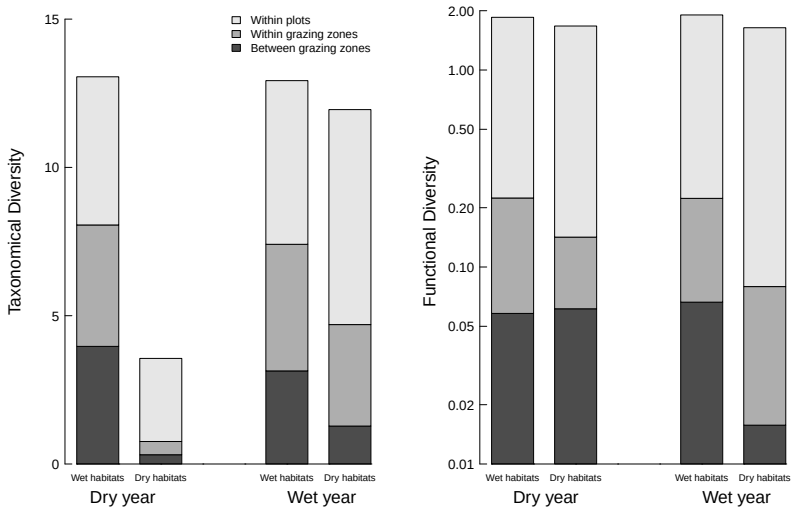

Figure 5.4. Partition of the effective number of species (Taxonomical diversity) and extent of index dissimilarity within communities (Functional diversity) at different levels of the sampling hierarchy. Note that the scale of the Functional diversity plot is log-transformed to facilitate its visualization.

5.5 CONCLUSIONS

The results indicate the importance of both functional differentiation and functional redundancy in species coexistence (Fukami *et al.* 2005) as well as their dependence on the combined effect of water availability and grazing regime. Despite great inter-annual variations in species diversity, functional diversity was often decoupled from species diversity and rather stable over time and space, showing the temporal functional stability of the system. However, under more limited water availability, grazing intensification reduced the functional diversity of these grasslands. The predicted reduction in rainfall in the Mediterranean area increases the risk of high grazing levels causing dramatic declines in the functional diversity of Mediterranean grasslands, probably compromising their stability and resilience over time. In the light of these results, we

suggest that overgrazing could have serious consequences for the functionality of these ecosystems, and therefore recommend the adoption of flexible grazing management schemes that should take species and functional diversities into account, and adapt the level of grazing pressure to water availability.

5.6 ACKNOWLEDGEMENTS

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Chapter 6

Grazing management or physiography? Factors controlling vegetation recovery in Mediterranean grasslands

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Abstract

Grazing intensification and abandonment are increasing the risk of degradation of Mediterranean grasslands. The development of techniques for monitoring grazing effects on herbaceous vegetation is an essential need for the management of these rangelands. However, the high variability of these systems make physiographical and management effects hard to disentangle and quantify. We present a methodology to support rangeland management and asses grazing effects on environmentally heterogeneous areas, and provide an example of its application in a Mediterranean rangeland in central Spain. We evaluated the difference in photosynthetically active vegetation cover between spring and summer using spectral mixture analysis of very high spatial resolution (2.4 m) Quickbird images. To analyze this difference, we developed Boosted Regression Trees models using grazing management (accumulated cost distance to points of livestock concentration and water points) and physiographical variables (slope, wetness, proximity to the closest tree and orientation). Results show that the main factor determining changes in vegetation cover is habitat type. The magnitude of this change maximized at intermediate grazing pressures for humid habitats, suggesting the existence of an optimal level of grazing in this zones, while in dry habitats differences in vegetation cover increased consistently along with grazing pressure. Our models provided a valuable insight into how different variables and its interactions affect the observed recovery capacity of vegetation. Moreover, by identifying areas in which grazing-induced land degradation could be taken place, our methodology can be used as a powerful tool in the management of highly heterogeneous rangelands.

6.1 INTRODUCTION

Extensive grazing is a fundamental factor in the shaping and maintenance of Mediterranean grasslands (Noy-Meir *et al.* 1989). Depending on productivity, grazing history and intensity, grazing can both increase or decrease grassland diversity and can be a significant agent of land degradation (Cingolani *et al.* 2005a; Thompson *et al.* 2009; Kleinebecker *et al.* 2011). In Mediterranean rangelands, soil fertility, water retention capacity and vegetation cover are enhanced at moderate levels of grazing (Peco *et al.* 2006). Nevertheless, when grazing intensity is too high, trampling and defoliation can reduce vegetation cover and increase the level of soil compaction, increasing the risk of soil erosion and productivity loss (Thornes 2007). Mediterranean environments are suffering an intensification process in the more productive areas, associated with the abandonment of others and environmental degradation (Ostermann 1998). Extensive grazing management practices are shifting to more intensive regimes, in which large-scale free range grazing is preferred over traditional shepherding, sheep are being substituted by cattle, and many extensive farms and transhumance practices are being abandoned (Peco *et al.* 2001). In this context, the development of techniques for monitoring the effects of grazing activities on vegetation is essential for rangeland management.

Assessment of the effects of grazing on vegetation and soil has traditionally been performed either by comparing farms with different grazing pressures (e.g. Peco *et al.* 2006) or by examining grazing gradients around water points. In the latter approach rangeland condition is assessed over broad spatial and temporal scales by relating vegetation cover with animal distribution patterns around water sources (Pickup and Chewings 1994). Remote sensing data fulfill the requisites of repetitiveness, objectivity and consistency and are observable over large areas (Diouf 2001; Röder and Hill 2009), being a very important tool for monitoring grazing effects (Kerr 2003). The main assumption of the grazing gradient technique, developed to be used in arid Australian rangelands, is that grazing effects decrease as distance from water increases. These effects are usually evaluated by inspecting the difference in vegetation cover between the dry and wet seasons. Grazing gradients remaining during the wet season are considered as indicators of permanent landscape degradation (Pickup *et al.* 1994; Harris and Asner 2003). The magnitude this difference is spatially irregular and largely depends on the

soil and physiographic characteristics of the site, and on the effect of disturbances like grazing. Thus, local grazing pressure can modify the response of vegetation after rainfalls for some given physiographic conditions (Pickup and Stafford-Smith 1993).

Mediterranean systems are characterized by a lower aridity, a lower relevance of watering points to establish grazing gradients (Röder *et al.* 2007) and a higher heterogeneity on physiographic and management conditions (Thornes 2007) than Australian ones. Thus, a different approximation has to be applied when studying grazing effects at landscape level in Mediterranean ecosystems. In order to perform this adaptation, Röder *et al.* (2007) used a cost surface model, which integrated the most important determinants of the spatial distribution of livestock and used woody vegetation cover to infer the grazing gradient. However, this adaptation only deals with woody vegetation, neglecting herbaceous vegetation, which is essential for grazing management. Herbaceous vegetation cover is hard to estimate at medium to low spatial resolution satellite imagery, because patches of woody vegetation are frequently mixed with herbaceous vegetation (Kuemmerle *et al.* 2006), but high resolution satellite images can be used to separate patches containing woody vegetation from those that only contain herbaceous vegetation.

There is a need of a deeper understanding of the processes that determine landscape patterns (Turner 1989), but the relative importance that physiographical and management variables and their interactions have on vegetation is hard to disentangle and quantify. Cost surface models can be used to infer grazing gradients, and statistical analysis can provide an estimate of the importance of these variables. Previous studies have used multiple regression approaches (Cingolani *et al.* 2008; Renison *et al.* 2009; Schulz *et al.* 2011), but the recent development of machine learning techniques provides new possibilities for studying these complex relationships. Among these techniques, Boosted Regression Trees (BRT) have proved to have a better predictive performance than traditional modeling methods. Their results are easily interpretable and provide a reliable identification of relevant variables and interactions (Elith *et al.* 2006; De' ath 2007).

The main objective of this study is to present a methodology to support rangeland management and assess grazing induced effects on vegetation cover on environmentally heterogeneous areas such as Mediterranean ones. We develop a method to disentangle the effects that phys-

iographic and management variables have on the changes in cover that herbaceous vegetation experienced between the wet and dry seasons of the year. As an example, we have applied the method to a Mediterranean rangeland in central Spain. Finally, we discuss the implications of the method for rangeland management and land degradation monitoring in highly heterogeneous semi-arid landscapes, as well as the relationships between the predictors and the observed recovery capacity of herbaceous vegetation.

6.2 MATERIAL AND METHODS

The study area occupies 1360 hectares and is situated 35 km north of Madrid (centre coordinate: 40°38′ N; 3°70′ W; mean elevation 860 m). Bedrock is composed of gneisses, and climate is typical Mediterranean, with very dry summers and maximum precipitation in spring and autumn. Annual rainfall is ca. 540 mm, with large interannual fluctuations, and mean temperature is ca. 13°C.

Clearing of the original forest, where *Quercus ilex* subsp. *ballota* and *Juniperus oxycedrus* were the dominant tree species resulted in a typical dehesa landscape with ca. 40 trees/ha. The estate has been extensively grazed for centuries and currently shelters approximately 600 cattle and 100 horses (0.51 Livestock units/ha). Three different habitat types can be distinguished: (1) humid and open habitats with deep soils, water and nutrient inflows and a very low density of woody species are located mainly along the low slopes and depressions; (2) dry habitats, characterized by shallow soils and nutrient and water outflows, located along the upper slopes; (3) tree islands associated with a single tree or a group of trees, which generally overlap with dry habitats and are characterized by increased shade and soil organic matter.

The grasslands are very species-rich (more than 20 species/400 cm^2) and mainly composed of annual species such as *Xolantha guttata*, *Leontodon taraxacoides* subsp. *longirostris*, *Hypochoeris glabra*, *Trifolium* spp., and the cryptophyte *Poa bulbosa*. The dominance of annual species causes a drastic reduction in herbaceous vegetation activity and cover during summer, because of the reduction in water availability.

Close to the centre of the study area, there is a shed and an important waterpoint, around which feedstuffs are usually supplied to animals. Because of this combination of factors, animals tend to gather in the proximity of that point of livestock concentration (PLC; Fig. A1).

In this study area we tested our methodology, consisting in: (1) calculation and mapping of the difference in herbaceous vegetation cover between the wet and the dry seasons of the year by means of a Linear Spectral Mixture Analysis (SMA); (2) development and validation of a cost distance model to infer the spatial pattern of grazing pressure; and (3) quantification of the effects of physiographic and grazing management related factors, as well as their interactions, on the recovery capacity of vegetation during the wet season (Fig. 6.1).

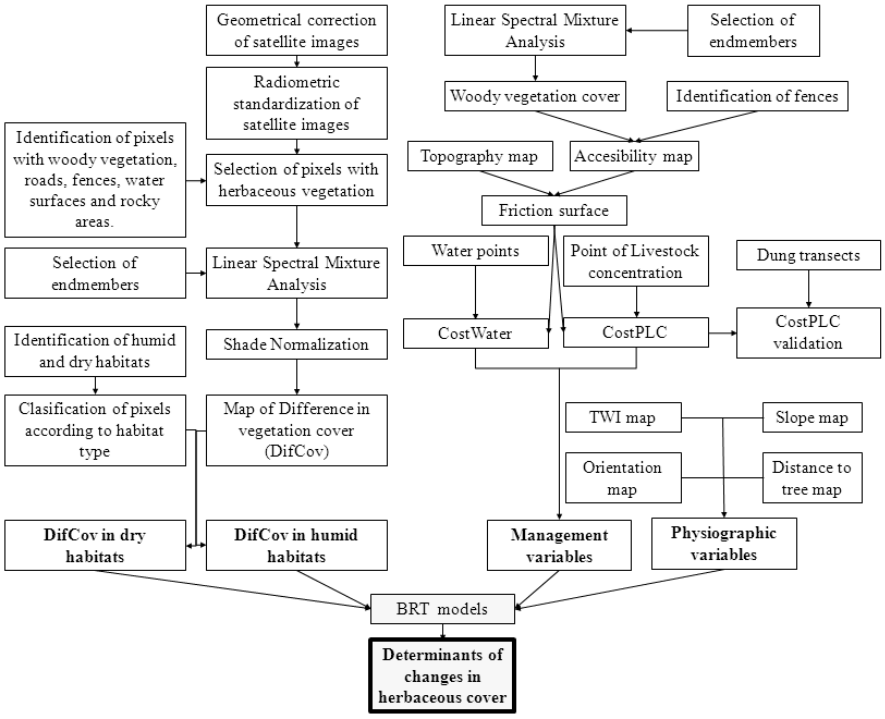

Figure 6.1. Flowchart representing the proposed methodology.

Image data and preprocessing

We used two Quickbird images, each consisting of one panchromatic image at 0.6*0.6 m^2 resolution and one multispectral image at 2.4*2.4 m^2 resolution, acquired 21 April 2002 and 28 July 2002, respectively. A digital elevation model (DEM) was created based on topographic maps at the scale of 1:5000 with 5 m contour lines. A set of aerial photographs of the area taken in 2001 at 0.3*0.3 m^2 resolution was also available. The images were geometrically corrected and registered to the Universal Transverse Mercator reference system (UTM; spheroid INT1909, datum ED1950, zone 30) using the DEM to correct for the topography-induced distortions, ground control points previously identified in the aerial photographs and the rational polynomial coefficients provided with the Quickbird images. Images Digital Numbers were converted to at-sensor radiance using the parameters provided with the images. In order to improve the comparability of the images, a relative radiometric normalization based on pseudo-invariant targets was applied to the April multispectral image. We applied a threshold to the aerial photograph in order to detect the pixels with presence of woody vegetation and resampled the resulting image to 2.4*2.4 m^2. We also identified and mapped roads, fences, water surfaces and rocky areas, and excluded them from the Quickbird images along with woody vegetation, obtaining two images showing only pixels covered by herbaceous vegetation. In order to ensure the complete removal of those features, we performed a one pixel buffer around them and eliminated those areas as well.

Since habitat type is expected to influence the effect of grazing on vegetation response (Bastin *et al.* 1993, Bardgett and Wardle 2003, Thompson *et al.* 2003), we classified pixels as belonging to dry or humid grasslands. Pixels located in open areas with a slope less than 10% were classified as humid habitats, and the rest as dry habitats, thus assigning all the pixels to any of the two habitat types (81,455 humid and 322,558 dry pixels; Fig. A2).

Differences in herbaceous vegetation cover

SMA (Smith *et al.* 1990) was used to infer the proportional amounts of Green Vegetation (GV), Soil and Shade for each pixel in the April and July multispectral images (Adams and Gillespie 2006). SMA assumes that the spectra can be modeled as a linear combination of two or more pure spectral references, the so-called endmembers (EM):

$$\rho_\lambda = \sum_{i=1}^{N} f_i * \rho_{\lambda i} + \epsilon_\lambda \quad and \quad \sum_{i=1}^{N} f_i = 1 \tag{6.1}$$

where ρ_λ is the reflectance of the mixed spectrum in band λ, $\rho_{\lambda i}$ is the reflectance of the endmember spectrum i in band λ, is the fraction of the endmember i, N is the number of endmembers and ϵ_λ is the residual error in band λ.

In addition to a zero-reflectance shade, two image EMs (photosynthetically active vegetation and soil) were manually selected by exploring the feature space of the four bands of the Quickbird images and according to previous knowledge of the area (Fig. A3). The reliability of the model was checked by examination of the RMSE and histograms of the fraction bands which were found to be in the range of 0-1 (meanRMSE$_{April}$=1.35%; meanRMSE$_{July}$=2.23%). Finally, shade normalization was carried out to account for albedo effects and generate physically meaningful fraction estimates.

The difference in cover between the wet and dry seasons for each pixel was calculated as the difference in GV cover in April and GV cover in July ($DifCov$):

$$DifCov = GV_{April} - GV_{July} \tag{6.2}$$

Given that these systems are largely dominated by annual species that spent the dry season (corresponding with the July image) as seeds, high values of $DifCov$ would indicate a better capacity to recover during the wet season (corresponding with the April image) than low values. This approach is very similar to the Resilience Method (Pickup *et al.* 1994), but the preeminence of annual species and the exclusion of pixels with presence of woody vegetation make unnecessary the correction for the vegetation cover existing in the dry season. Thus, values of $DifCov$ lower than those expected under determined physiographic conditions could be interpreted as indicators of elevated effects of grazing.

Cost surface modelling

Livestock in the study area spend a significant amount of time in the PLC close to the centre of the estate. Besides that main PLC, there are several water points around which animals tend to gather (Fig. A1). To estimate grazing pressure, we calculated the accumulated costs of travelling

away from the PLC (CostPLC) and from the nearest water point (CostWater) to each pixel using the Cost Distance algorithm implemented in the sofware ArcGIS 9.3 (ESRI 2008; Fig. 6.2). Our model considered two factors determining livestock distribution: topography and accessibility.

Topography

The slope of the pixels in the DEM of the study area ranged from 0% to 54% (Fig. A4). Slope is an important factor because cattle and horses avoid slopes exceeding 20% and 30% respectively, and cattle tend to travel through areas with less slope than average in rugged terrain (Ganskopp and Vavra 1987; Ganskopp *et al.* 2000). Similar to previous works on cost surfaces (Röder *et al.* 2007; Paudel and Andersen 2010), slope values were first converted to degrees and then to cost surface (0-100) as:

$$C_{slope} = 100 * (slope/90), \tag{6.3}$$

Thus, the component of the friction due to slope for a pixel with 0° slope was 0, while it was 100 for a pixel with a 90°slope.

Accessibility

This factor had two parts: the first one was related with the presence of fences inside the range. Fences were mapped and excluded, thus not allowing movement across pixels containing a fence. The second part of the factor was related to woody vegetation density. In order to fulfill the realistic assumption that livestock would go around patches with a high

Table 6.1. Explanatory variables selected.

Variable	Description	Mean
PHYSIOGRAPHIC		
Slope	Average slope derived from DEM layer (%)	10.30
TWI	Topographic Wetness Index (see text for further explanation)	6.46
Aspect	Dominant exposure of the pixel: N = North, E = East, S = South, W = West.	
DistTree	Distance to the closest pixel with presence of woody vegetation (m)	3.89
MANAGEMENT		
CostWater	Accumulated cost distance to the closest water point (see text for further explanation)	4014
CostPLC	Accumulated cost distance to the main Point of Livestock Concentration (see text for further explanation)	6325

cover of woody vegetation rather than crossing them. In order to estimate the cover of woody vegetation a new EM model was carried out in the July image only for the pixels with woody vegetation (Fig. A3). We mapped percentage of cover of woody vegetation for the pixels with presence of woody vegetation (Fig. A5). In order to match the largest value of this parameter (i.e. a 100% cover of woody vegetation) to that corresponding to the maximum slope in the estate (ca. 24°), this percentage was multiplied by 0.25 and the result was added to each pixel friction value.

Validation

On April 2009 and December 2011, we sampled 80 points to test for the validity of the cost distance gradients as indicators of grazing pressure. 40 of these points, distributed along the gradient of distance to the main PLC, distant at least 1500 cost distance units from the closest water point, were used to validate the CostPLC gradient. The other 40 were at least 2000 cost units away from the main PLC and were used to validate the CostWater gradient. On each point, we located four 30 x 2 m transects, oriented towards the N, W, S and E directions respectively. On each meter along the axis of each transect we registered the presence or absence of excrements bigger than 3 cm. For each point we averaged the values of its four transects as an estimator of dung cover. Dung estimations are good indicators of the relative abundance of livestock and have been widely used to estimate grazing pressure along grazing gradients (e.g. Riginos and Forman 2003). Finally, we fitted a lowess model (Cleveland 1979) to examine the relationship between cost distance and dung cover.

Factors determining variability in *DifCov*

Vegetation response can depend on many factors such as soil type, slope, water availability, orientation or distance to the closest tree. Besides, grazing pressure (measured in this paper as cost distance to the main PLC and cost distance to the closest water point) has been found to be correlated with vegetation response (Todd 2006; Röder *et al.* 2007). Six explanatory variables, available as GIS data for all the study area, were chosen for being reasonably relevant in order to determine the differences in herbaceous cover between the wet and dry seasons (Table 6.1). Predictors were classified either as physiographic or grazing management related predictors.

To estimate water availability for each pixel, we used the Topographic Wetness Index (TWI; Beven and Kirkby 1979). TWI, defined as $\ln(a/\tan\beta)$, were a is the local upslope area draining through a certain point per unit contour length and β is the local slope angle (Fig. A6), is known to be a good predictor of important soil features such as moisture, pH and groundwater level (Sørensen *et al.* 2006).

We also calculated for each pixel the linear distance to the closest pixel with presence of woody vegetation and used this measure as an indicator of the distance to the closest tree. Trees can compete with understory plants for resources, but they can also provide an enhancement in environmental conditions for herbaceous vegetation (see review in Marañón *et al.* 2009).

To explore the relationship between the explanatory variables and $DifCov$, we fitted BRT models for each habitat type. BRT models can deal with complex interactions and nonlinear relationships and are considered to have a superior predictive performance compared to traditional methods (Elith *et al.* 2006; Elith *et al.* 2008). In this technique, successive regression trees are fitted and sequentially merged. The first tree is the one that best explains the original data and following ones are fitted to the residuals of the previous models without affecting them. A random subset of the data is used on each subsequent regression tree, thus improving predictive performance of the final model. Besides the number of trees that form the BRT, a number of parameters have to be fixed when constructing these models. First, the proportion of the data randomly selected in each subset is given by the bag fraction parameter. Second, the learning rate determines the contribution of each tree to the final model: smaller learning rates provide higher prediction performance, but substantially increase computation time. Finally, the tree size parameter determines the number of splits of the individual trees. High values of this parameter allow to fit complex interactions between predictor variables. The relative influence of each predictor to the final model is calculated by averaging across all trees the contribution of each predictor.

BRT models were fitted in R (v 2.12.2, R Development Core Team 2011) using the package gbm (Ridgeway 2010). Given the large size of the database, we randomly selected a training subsample of 10,000 pixels for each habitat (one for humid and one for dry areas) to fit the models, and used the rest of the data of the respective habitat to test the fit of the models. For each habitat, we fitted three BRT models with tree sizes of

1, 3 and 5, respectively. Since the number of trees and the learning rate are correlated, we tested several learning rates for each model using the code and recommendations provided by Elith *et al.* (2008). Their algorithm finds the optimal number of trees by using k-fold cross validation. We chose the smallest learning rate that yielded an optimal number of trees below 10,000, in order to keep computation time within reasonable limits.

The variance explained by each model on the respective testing subsample was the criterion used to select the best values for tree size. To keep the model as simple as possible, whenever a variable had a relative influence smaller than 2%, the model was refitted without this variable and the simplest model was preferred when the reduction in explained variance on the testing subsample was less than 5% compared to the full model.

In order to provide confidence intervals of the predictions and fitted functions of the models with the chosen parameters, we made 100 BRT models, each fitted to a different random subsample of 10,000 pixels. Finally, for the chosen models, all pairwise interactions between predictors were plotted, and their relative strengths calculated using Friedman's H-statistic.

6.3 RESULTS

The lowess models revealed a relationship between cost distances (CostPLC and CostWater) and dung cover consistent with the expected in a grazing pressure gradient. This confirms the idea that the main PLC and the water points are the main determinants of grazing pressure in the area, supporting the validity of the cost surface modeling approach to infer grazing pressure and the posterior use of CostPLC and CostWater in the BRT models as management indicators (Figs. 6.2 and 6.3).

Examination of the $DifCov$ map revealed that the main factor determining differences in vegetation cover is the habitat type in which it is located (Fig. B1). $DifCov$ was consistently higher on humid areas in contrast with lower but more variable values on dry areas (means: 55.54% and 41.77%, respectively).

Humid areas

Learning rate selected was 0.05 for the three tree sizes. Orientation was not retained in any model because of its low predictive capacity. The

model fitted with a tree size of 5 had the best predictive capacity for independent data (45.96% of the variance for independent data), and was selected for being fitted repeatedly with different subsets of the data (Table 6.2).

Management predictors had a higher relative importance than physiographic ones. CostWater was the most important predictor regardless of tree size, though its relative importance decreased as tree complexity increased (Table 6.3). Differences in vegetation cover increased abruptly in the first meters nearby water points which would correspond to the '*sacrifice zone*' (Thrash and Derry 1999) and kept increasing until distances of 4,000 cost units. Beyond that distance, a decrease in $DifCov$ was observed. Predicted $DifCov$ tended to increase with distance from

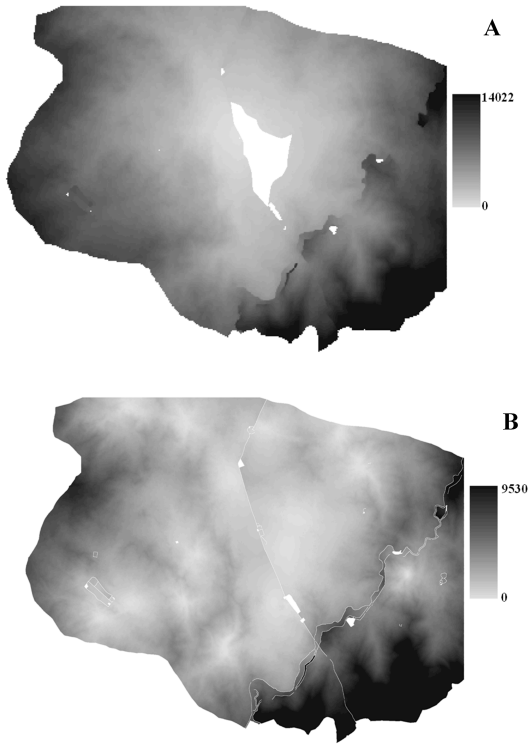

Figure 6.2. Accumulated cost distance surfaces with (A) the main PLC and (B) water points as starting features.

the main PLC, peaking at around 10,000 cost units and declining after that point. Vegetation response was predicted to raise rapidly within the first meters of distance to the closest tree and stabilized beyond 15 meters. $DifCov$ increased throughout the whole range of TWI. A small peak in vegetation response was found for values of slope close to 5% (Fig. 6.4A).

Among the pairwise interactions between the different variables, the one between CostWater and CostPLC was by far the most important of all (Friedman's H = 0.63; Fig. 6.5).

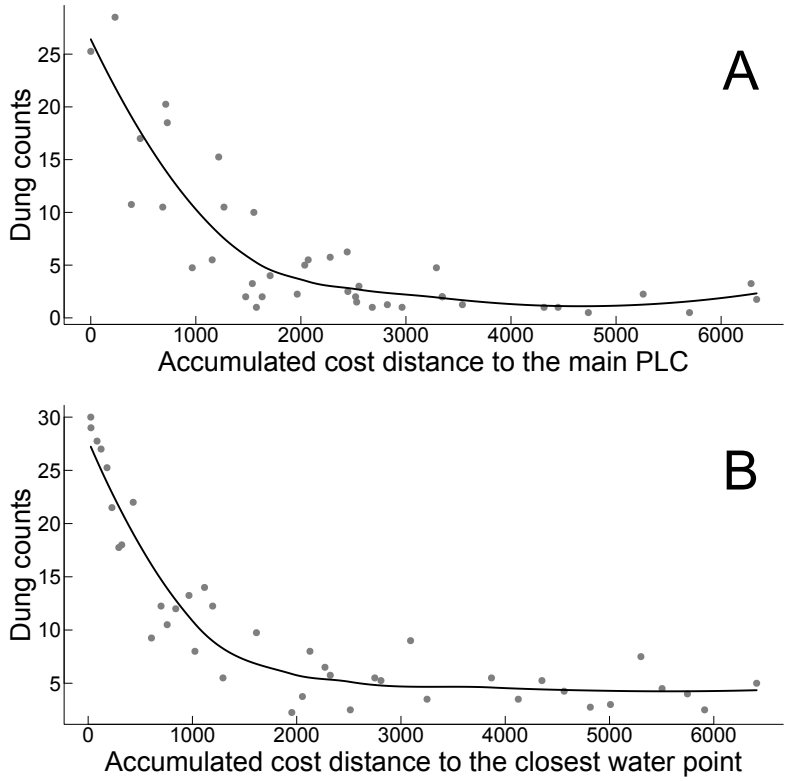

Figure 6.3. Lowess model showing the relationship between (A) accumulated cost distance to the main PLC and dung cover and (B) accumulated cost distance to the closest water point and dung cover.

Dry areas

The best value for learning rate was 0.005, independent of tree size. As well as in humid areas, Orientation was not selected as a significant predictor in any model. The model with a tree size of 5, which explained a 15.26% of the variance for independent data, was selected. Distance to the closest tree was the most important factor in the model, and the shape of the response was similar as for humid areas. $DifCov$ response to CostWater was also comparable to the one for humid areas in the first part of the range, but $DifCov$ did not decrease with higher values of this parameter. This continuous increasing response was also observed for CostPLC, and this increase was particularly strong in the most distant points from the main PLC. Predicted values of $DifCov$ peaked at slope values around 5% and constantly decreased beyond that point. The re-

Table 6.2. Summary of the models fitted with different tree sizes for each habitat.

Tree size	Learning rate	Ntrees	Variance explained (%)
HUMID AREAS			
1	0.05	4000	26.48
3	0.05	6600	38.37
5	0.05	8000	45.96
DRY AREAS			
1	0.005	7200	12.56
3	0.005	7400	13.38
5	0.005	5600	15.26

Table 6.3. Relative influence of the selected predictors on each model.

Variable	*Tree Size*			Average
	1	3	5	
HUMID AREAS				
CostWater	39.00	28.86	28.53	32.13
CostPLC	23.48	26.98	25.77	25.41
Slope	12.44	16.03	16.41	14.96
TWI	12.35	15.43	17.05	14.94
DRY AREAS				
DistTree	40.40	23.85	20.43	28.23
CostWater	19.01	23.34	20.98	21.11
Slope	19.06	18.07	21.53	19.53
TWI	12.90	17.48	18.31	16.23
CostPLC	8.62	17.26	18.74	14.87

sponse to TWI showed a monotonic increase throughout the whole range of the predictor (Fig. 6.4B).

The effect of interactions between predictors was much smaller than for humid areas, the most important being again the interaction between CostWater and CostPLC (Friedman's H = 0.50).

6.4 DISCUSSION

Our study provided evidence for the influence of grazing on the changes in cover of herbaceous vegetation in Mediterranean environments and for the strong interactive effects of management and physiographical variables. The direction of the effects and the relative importance of these variables differed between habitat types, suggesting that vegetation responses to grazing in the study area were strongly modulated by the productivity level.

As expected, habitat type was the most important determinant of vegetation cover. Although the mean vegetation cover in summer was slightly higher for dry habitats than for humid ones (23.89% vs 20.01%), the differences in cover between the wet and the dry seasons were significantly higher in humid habitats. This result is consistent with other studies in which water availability had a greater influence than grazing on the response of vegetation (Biondini *et al.* 1998; Schönbach *et al.* 2010).

The main finding of this study is the interactive effect of habitat type and grazing intensity. In humid areas, we observed that differences in vegetation cover maximized at intermediate levels of grazing pressure and were smaller for the highest and lowest extremes of the grazing gradient (Figs. 6.4A and 6.5). On the other hand, in the dry habitats these differences consistently increased as grazing pressure decreased (Figs. 6.4B and 6.5). Humid habitats have deeper soils and higher water availability than dry ones, which facilitate plant establishment when there is enough water available and thus, enhance vegetation recovery (Bastin *et al.* 1993). Livestock activity in semi-arid environments, especially trampling and defoliation, is known to reduce water availability for plants, because of the increased run-off and reduced water infiltration caused by the reduction of vegetation cover and compaction of soils (Thornes 2007; Schacht and Reece 2008). Several authors have described that ecosystem processes in grasslands are determined by the complex interactions between grazing pressure, soil fertility and water availability (Shariff *et al.* 1994; Biondini *et al.* 1998; Bardgett and Wardle 2003).

In our case, water availability seems to play a strong role in modulating plant-herbivore interactions. In humid habitats intermediate levels of grazing promoted the recovery capacity of herbaceous vegetation, which coincides with the grazing optimization hypothesis that predicts an increase in aboveground net primary production (ANPP) at moderate grazing pressures (McNaughton 1979). However, in dry areas grazing was negatively correlated with differences in cover between the wet and dry seasons. Since satellite observations of vegetation indices and primary production are strongly correlated (Prince 1991), our results show that the response of ANPP to grazing could be contingent on productivity, ranging from positive to negative responses depending on water availability. This is consistent with the results reported by Augustine and McNaughton (2006), who described positive effects of moderate grazing pressures on ANPP of a semi-arid savannah in an above-average rainfall year and negative effects during a dry year. Similarly, Blanco *et al.* (2008) showed a similar interactive effect between grazing pressure and water availability on ANPP across an aridity gradient.

The effect of physiographical variables was similar in both types of habitats. As expected, the differences in cover were positively correlated with TWI. The biggest marginal increases within both habitat types were found for the dryer areas, indicating that small increases in water availability for plants were more important in dry conditions and that beyond some threshold (TWI values between 4 and 5; Fig. 6.5) these increases did not improve significantly the recovery capacity of the vegetation.

Differences in cover also increased along with the distance to the closest tree. This increase was especially important in the first 5 m from the closest tree, and almost disappeared for distances bigger than 15 m. Trees are known to play an important role in Mediterranean oak savannas and their effects on the herbaceous vegetation can range from positive to negative (Marañón *et al.* 2009). In our case, trees reduced the differences in herbaceous cover in the areas surrounding them, because of the relatively high cover that was observed in these areas during the dry season. This softening on the reduction in vegetation cover that takes place in the dry season effect, probably caused by the increased shade in the proximity of the trees (Moreno Marcos *et al.* 2007), emphasizes the importance of trees as a fundamental feature in dehesa systems (Manning *et al.* 2009).

Slope had a much more clear effect in dry than in humid habitats, because of the much bigger range of slopes found in dry areas. For slopes above 10%, $DifCov$ was negatively correlated with slope. This

was surely because of the increased runoff and erosion experimented by soils with high slopes. However, the maximum $DifCov$ was found for areas with slopes in the range of 5-8%, and not in the completely flat ones, which could be caused by the preference of cattle for these flat areas (De Miguel *et al.* 1997).

Management implications

The methodology presented in this paper allows the use of the grazing gradient method to study rangelands with highly heterogeneous conditions. Previous studies have used satellite images with a much coarser resolution (Bastin *et al.* 1996; Harris and Asner 2003; Cingolani *et al.* 2008; Paudel and Andersen 2010), but the recommended pixel size for Mediterranean environments, characterized by an intimate mixture of woody and herbaceous patches, is 6 m or less (Rahman 2003). The exclusion of pixels with woody vegetation, an essential step in order to eliminate the prerequisite of correcting for the woody cover in the calculation of the difference of herbaceous vegetation cover between spring and summer (Bastin *et al.* 1996) can only be done using the appropriate resolution. Therefore, the use of an adequate pixel size is crucial for the extension of the grazing gradient method illustrated in this paper.

The use of machine learning techniques such as BRT provides a strong predictive capacity, making it easier to simulate the effects of alternative management decisions such as the creation or suppression of fences, water points or livestock infrastructure and those of actions like the clearing of woody vegetation. Besides this, the graphical output of the model allows to explore the effects of the individual variables as well as their pairwise interactions, offering a valuable knowledge on the functioning of these ecosystems. In particular, careful interpretation of the interaction plot (Fig. 6.5) can be very useful in range management. In the study area used as an example in this paper, it allowed to discover some very interesting patterns, like the thresholds found for the variables TWI and DistTree, or the grazing pressures at which $DifCov$ maximized in humid habitats. Along with the use of the interaction plot, mapping the residuals of the models (i.e. , the difference between the observed and predicted values of $DifCov$; Fig. B2) can help to expose areas where the difference in cover of the herbaceous vegetation is lower than expected, revealing that some factor is negatively affecting this capacity. In the provided example we found some of these areas. Two corresponded to

points in dry habitats with very high livestock concentrations because of the supply of feedstuffs. Many of the other areas with a $DifCov$ below expected were close to roads. This happened despite the exclusion of pixels within five meters from the road edges, and is probably because of the combination of two factors. First, the low quality of many of these roads makes them impassable after rainfalls very often, which force managers to drive over the grass, degrading these areas. Second, the enhanced accessibility of the areas close to roads probably increases the likelihood of livestock to use them. These findings could be easily translated into management prescriptions. Actions likely to increase the grazing pressure in dry areas, such as the creation of new water points, new roads or changes in areas in which feedstuffs are provided to animals must be studied with special care because of its possible negative effects on the conservation status of the farms.

Some improvements can be made in future applications of the technique. For example, it could be applied to estates with more than one PLC, just by taking into account data relative to the number of animals using each point (e.g. Paudel and Andersen 2010). Another possible improvement would be to quantify the feeding preferences of the animals and include them in the cost surface calculation, as in Röder *et al.* (2007).

6.5 CONCLUSIONS

The application of cost surface modeling along with BRT provides an effective methodology for identifying the drivers of changes in vegetation cover between the wet and dry seasons in heterogeneous environments. Our approach provides a valuable insight into which areas are more susceptible to experience grazing-induced land degradation and facilitate the adoption of the necessary restoration or preventive measures to solve the potential problems associated with this process. Thus, it can be applied as a powerful tool in the management of highly heterogeneous rangelands.

Among the studied, the most important factor determining the extent of the differences in vegetation cover and modulating the effect of the management variables was water availability. This results agree with those of previous studies in the same area that found that the effects of grazing on the diversity of plant communities are strongly mediated by water availability, ranging from a reduction in diversity in dry conditions to an increase in wet conditions (Carmona *et al.* 2012). The different

responses to grazing between humid and dry habitats can have deep implications for the grazing management of these systems. A reduction in vegetation cover can increase the run-off and reduce the soil water infiltration capacity, initiating negative feedbacks that would induce land degradation in dry habitats (Rietkerk *et al.* 2000). The predicted reduction in rainfall in the Mediterranean area in the coming years increases the likelihood of these processes to occur in the study area, especially in dry habitats, underlining the importance of the development and systematic application of rangeland monitoring methodologies like the one presented in this paper.

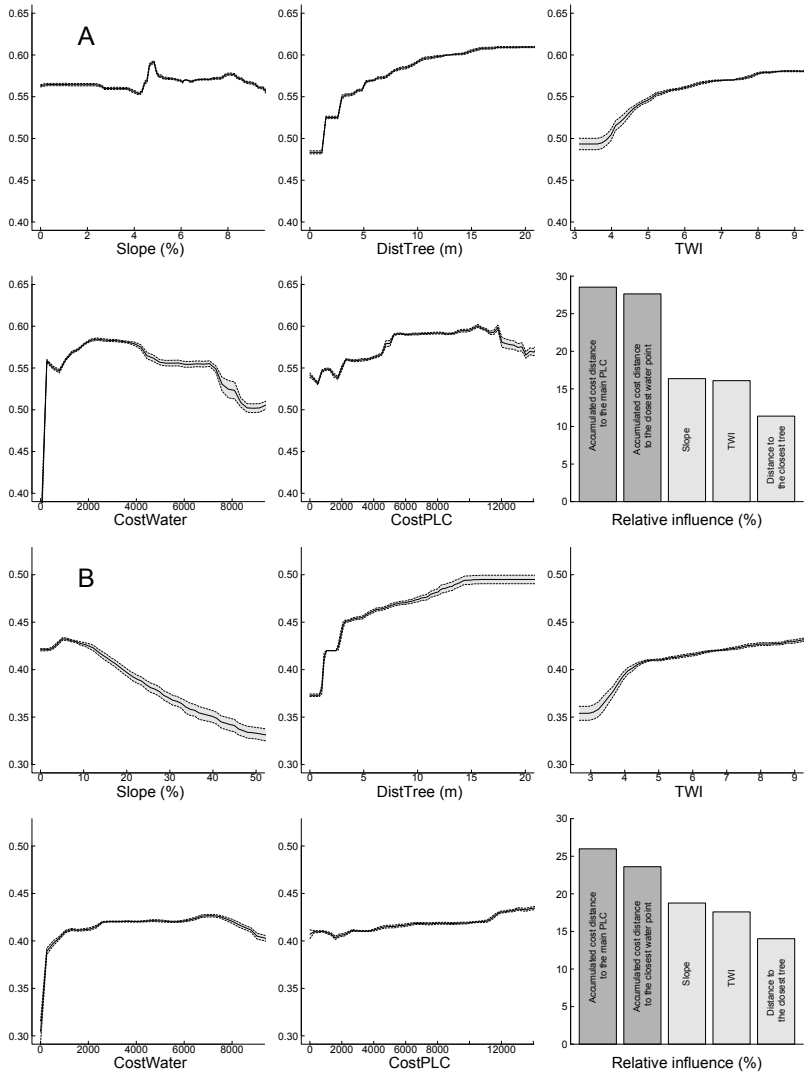

Figure 6.4. Mean (solid line) and 95% confidence intervals (dashed lines and shaded areas) of the predicted values of difference in vegetation cover between April and July calculated by 100 boosted regression tree models with a tree size of 5 for (A) humid and (B) dry habitats. Each model was fitted to a different subsample of 10,000 pixels of the original dataset. All other variables are held at their means. The last plot for each habitat shows the average relative influence of each predictor on the fitted models, with management variables in dark grey tones and physiographical variables in light grey tones.

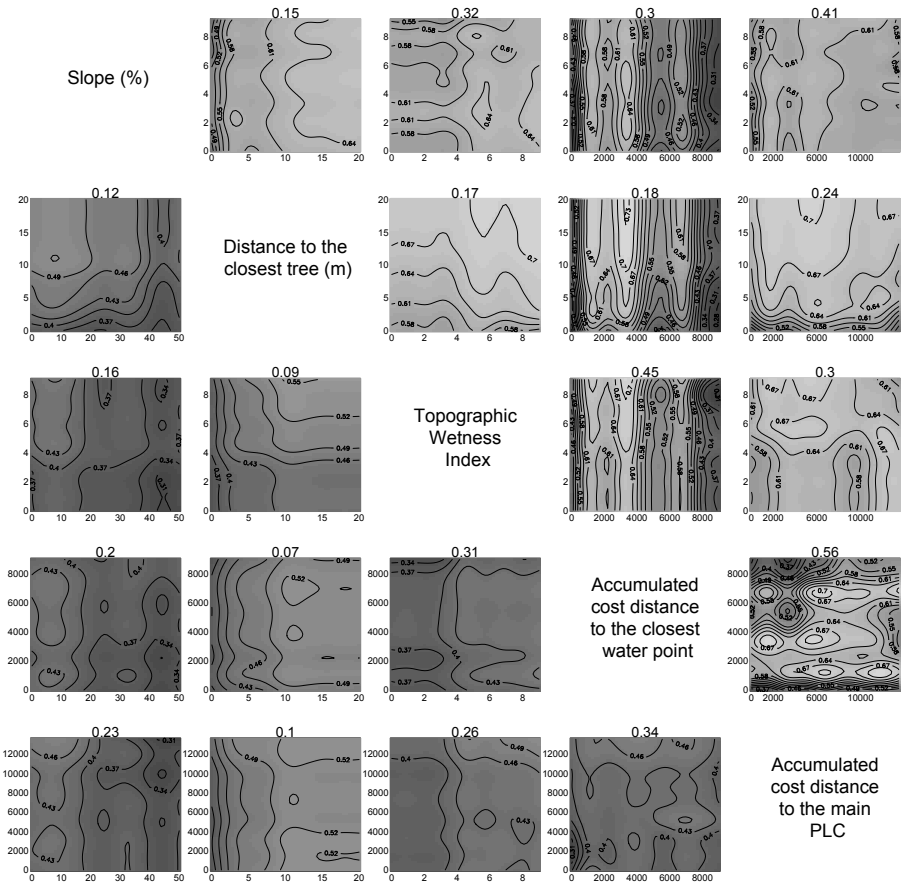

Figure 6.5. Partial dependence plots for the interactions between all predictors for boosted regression tree models with a tree size of 5 for humid areas (above the diagonal) and dry areas (under the diagonal). The predictor variable in the ordinate axis of each plot is named in the rows and the predictor variable in the abscissa axis is named in the columns. All other variables are held at their means. Brighter tones represent high values of $DifCov$ and darker tones represent low values of $DifCov$. Friedman's H-statistic is shown above each plot to indicate the importance of the interaction.

6.6 ACKNOWLEDGEMENTS

Financial support was received from the Spanish Ministry of Science (Projects CGL2007-63382 and CGL2011-24871), the Madrid Regional Government (Projects REMEDINAL-S0505/AMB-0335 and REMEDINAL2-S2009/AMB-1783), and the Spanish Ministry of Education (FPI grant BES-2008-009821 for CPC and Salvador de Madariaga mobility grant PR2011-0491 for BP).

6.7 SUPPORTING INFORMATION

Additional supporting information for this article:

Appendix A.

Suplementary information.

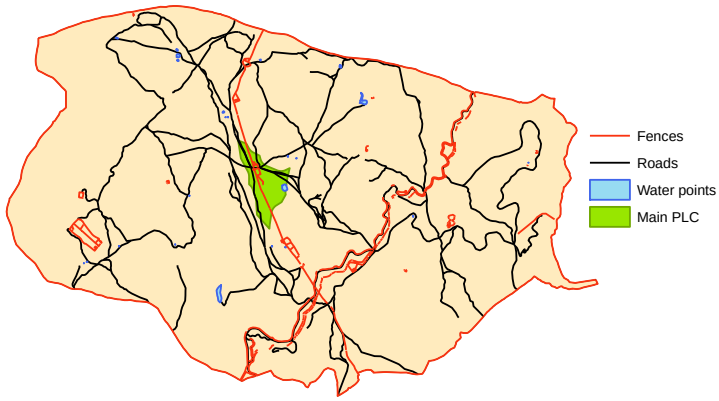

Figure 6.6. Figure A1. Map of the estate in which the methodology explained in the paper has been applied. Important features such as the water points and the position of the main point of livestock concentration (PLC) are shown.

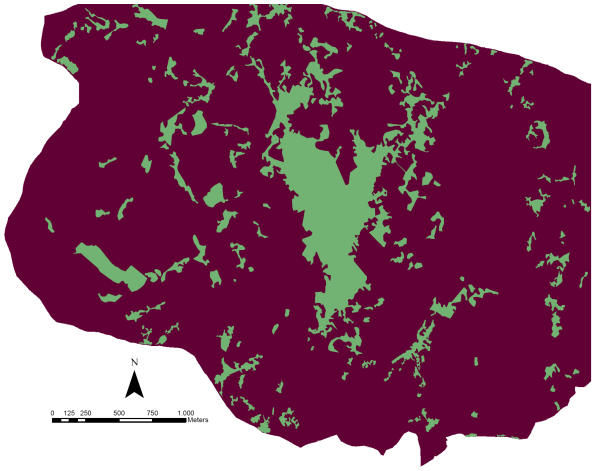

Figure 6.7. Figure A2. Location of the two habitat types considered in the study. Humid habitats are represented in green and dry habitats in red.

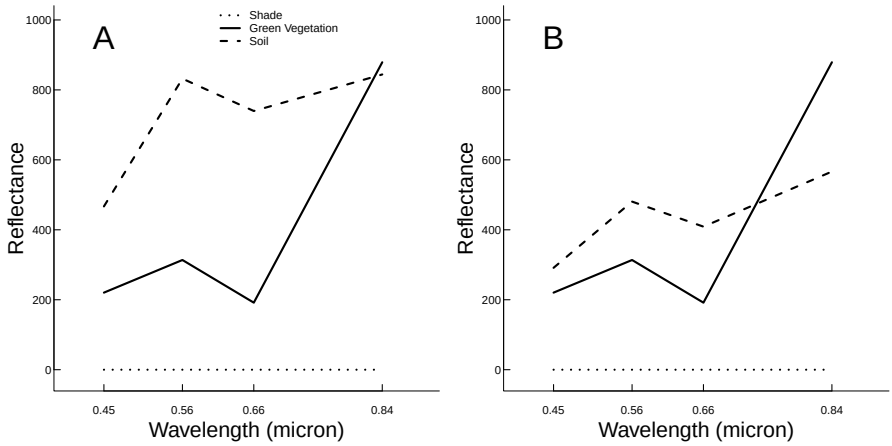

Figure 6.8. Figure A3. Endmember models selected in the Linear Spectral Mixture Analysis for (A) pixels with no woody vegetation and (B) pixels with woody vegetation.

Figure 6.9. Figure A4. Slope percentages in the study area.

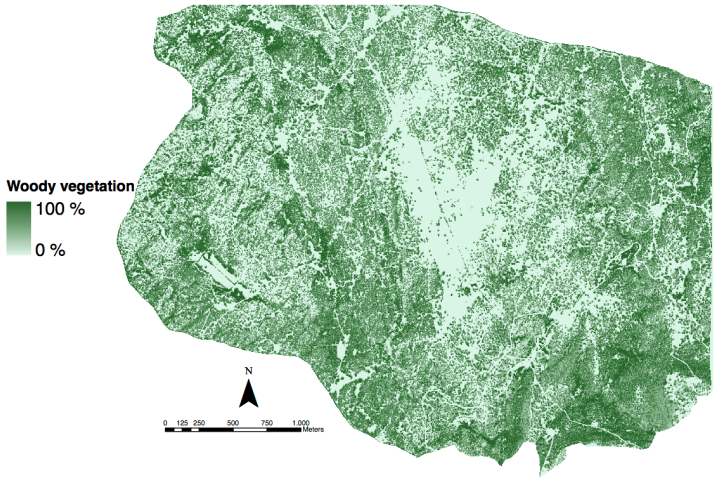

Figure 6.10. Figure A5. Percent cover of woody vegetation in the study area.

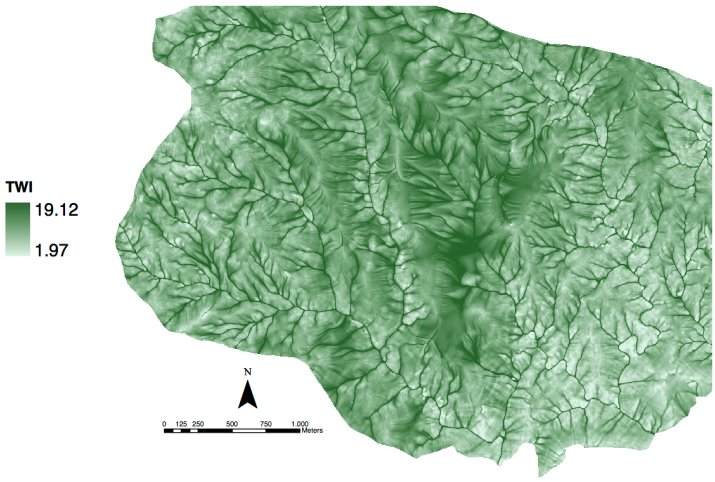

Figure 6.11. Figure A6. Topographic Wetness Index in the study area.

Appendix B.

DifCov and BRT models predictions.

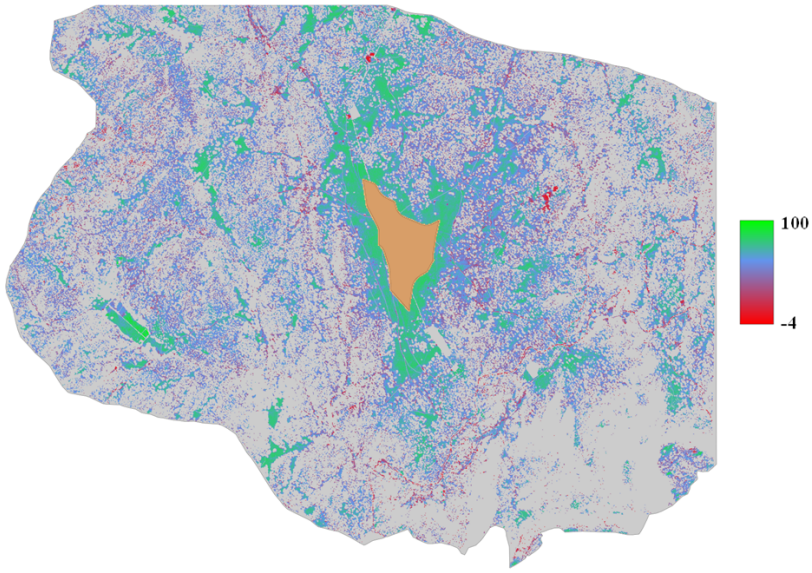

Figure 6.12. Figure B1. Difference in percentage of vegetation cover between April and July. Green tones indicate high differences while red ones indicate small differences. Pixels with presence of woody vegetation are shown in grey. The main PLC is represented in orange.

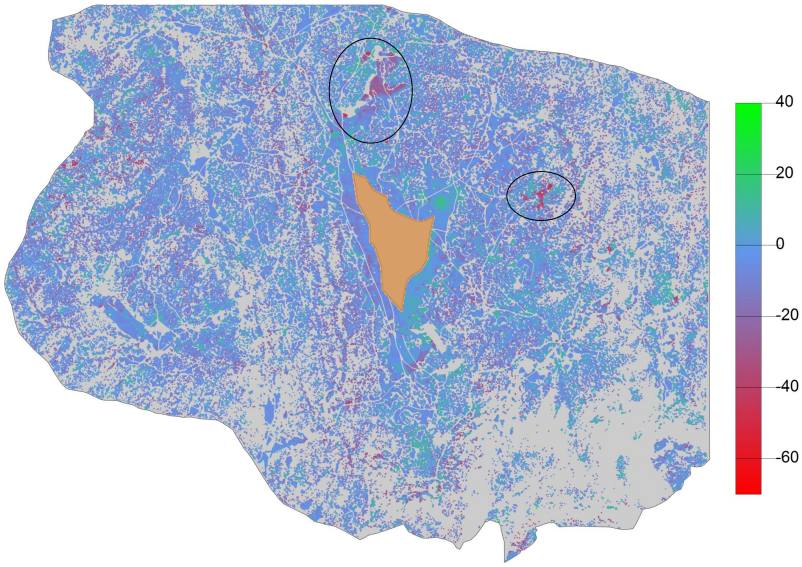

Figure 6.13. Figure B2. Difference between the observed and predicted percentages of $DifCov$. Green tones indicate that observed $DifCov$ is above the values predicted by the BRT models and red ones indicate that $DifCov$ is below predicted values. Circles indicate areas where vegetation recovery capacity is especially reduced because of increased grazing pressure caused by the supply of feedstuffs. The main PLC is represented in orange.

Chapter 7

Assessing the effects of seasonal grazing on holm oak regeneration: implications for the conservation of Mediterranean dehesas

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Abstract

Scattered trees in agricultural landscapes are globally declining due to the intensification of agricultural practices. Dehesas, highly diverse Mediterranean open woodlands, are seriously affected by this problem, caused by a generalized regeneration failure of oaks, which compromise their long-term stability. Dehesas traditionally were the wintering areas for transhumant herds, but transhumance is disappearing in the Mediterranean, due to agricultural intensification. Reductions in grazing intensity or grazing abandonment have been proposed to improve oaks regeneration in dehesas, but the effect of the recovery of non-continuous grazing practices such as transhumance has not been tested to date. We measured three specific aspects of holm oaks regeneration and condition (browsing status, regeneration and size structure) in dehesas under transhumant grazing and in dehesas under permanent grazing in southern Spain. Oak juveniles were remarkably less browsed and occupied a much higher surface in transhumant than in permanently-grazed estates. As a consequence, the density of saplings was higher in transhumant estates. Despite transhumant grazing is necessarily associated with a reduction in the stock capacity, the timing of grazing was always included as a predictor in the best models to explain the condition and density of the holm oaks. Our results suggest that the lack of oak regeneration in dehesas can be caused not only by the increases in stocking rates, but also by the recent abandonment of traditional grazing practices like transhumance. We propose the recovery of seasonal grazing regimes based on transhumant pastoralism as a measure to improve the conservation status of dehesas.

7.1 INTRODUCTION

Scattered trees in agricultural landscapes are widely recognized as keystone structures because of the large number of ecological functions that depend upon their presence (Fischer *et al.* 2010; Manning *et al.* 2006), but they are globally declining due to the intensification of agricultural practices (Gibbons *et al.* 2008). Multifunctional agrarian social-ecological systems, where the interaction between human populations and nature has shaped landscapes for millennia, are largely responsible for the high biodiversity of the Mediterranean Basin (di Castri 1981). Among these landscapes, dehesas, Mediterranean open woodlands resulting from the clearing of original evergreen oak woodland and shrubland areas (Plieninger 2007), also known as montados in Portugal, constitute an example of agrosilvopastoral ecosystems with a high biodiversity and have been widely presented as the paradigm of a sustainable land-use (eg. Pinto-Correia *et al.* 2011). These systems occupy a large surface in the Iberian Peninsula and its conservation is considered very important for both environmental and socio-economic reasons (Moreno and Pulido 2009). Among the many production activities that take place in dehesas, livestock production is the most important, especially in those dominated by holm oaks (*Quercus ilex* subsp. *ballota*; Pinto-Correia and Mascarenhas 1999; Pinto-Correia *et al.* 2011). Traditionally, dehesas have been grazed by domestic livestock, mainly sheep and cattle, at low stocking rates (Peco *et al.* 2006). Livestock feed on the acorns and grass under the canopy of semiopen woodland, which also provides firewood, hence the trees are viewed as an integrated part of the system, and as a result are managed and regularly pruned (Blondel 2006).

Mediterranean climate is characterized by marked differences between wet winters and dry summers with great inter-annual variability in rainfall, which have clear effects in the productivity of its grasslands. This, along with the high variability in topographic conditions, makes transhumance (seasonal movements of livestock) a successful grazing management practice in these environments (Manzano and Casas 2010; Niamir-Fuller and Turner 1999; Weber and Horst 2011). Transhumance practices consist in a regular and periodic movement of livestock from winter pastures (valley bottoms and/or southern latitudes) to summer pastures (mountains and/or northern latitudes; Weber and Horst 2011), optimizing the use of the existing resources (Ruiz and Ruiz 1986). Because of its size, climate, topography and anthropological characteris-

tics, transhumance systems have historically had a great importance in the Iberian Peninsula (Manzano and Casas 2010; Ruiz and Ruiz 1986).

For more than a century, European agricultural systems have been facing intensification of agricultural practices in the more productive or accessible areas, together with abandonment in the marginal ones (Ostermann 1998, Stoate *et al.* 2009). Extensive grazing management practices are being substituted by intensive regimes, which include the preference for large-scale free range grazing over traditional shepherding (Peco *et al.* 2001). As part of these processes, and despite its adaptive nature and wide distribution in the past, transhumance has been disappearing in the Mediterranean, especially over the last 50 years. Several social and economic factors lie behind this negative tendency; one of the most important reasons is society's failure to acknowledge the wide range of Ecosystem Services provided by this traditional practice and the managed ecosystems maintained by transhumant livestock (Oteros-Rozas *et al.* 2012). Dehesas, that have traditionally been the wintering areas for transhumant herds, are currently facing deterioration partly because of the overexploitation of estates, which is somewhat related with the sedentarization of previously transhumant herds.

Similarly to other landscapes composed by scattered trees, the last decades have seen a remarkable decrease in the total area covered by dehesas as well as in the tree densities of the remaining ones (Moreno and Pulido 2009). Studies on the size structure of dehesas have shown that these systems are characterized by over-aged oak stands, because of a disruption in the forest cycle characterized by an extensive lack of regeneration (Montoya 1998; Plieninger *et al.* 2003; Plieninger 2007). Whether the lack of regeneration of trees in dehesas is caused by recent intensification processes or is an inherent feature of grazed dehesas independent of grazing intensity is a question that has not been definitely answered to date (Moreno and Pulido 2009; Plieninger *et al.* 2011). Regardless of its causes, lack of regeneration of the tree layer is threatening the long-term stability of dehesas, because of the capital importance that oaks have on these systems (Joffre *et al.* 1999; Plieninger *et al.* 2003).

Consequently, there is an urgent need to develop measures that would improve the regeneration of oaks in dehesas (Gibbons *et al.* 2008; Pinto-Correia *et al.* 2011). Several options have been proposed to achieve this objective, such as reductions in grazing intensity, afforestation or grazing abandonment. However, the implementation of these options is not possible without severe ecological and socio-economic costs. For

example, the drastic reductions in the stock capacity that would be necessary to achieve a carrying capacity compatible with the regeneration of holm oak would be associated with very high socio-economic costs for farmers (Plieninger 2007). Furthermore, a total abandonment of grazing would increase the fire risk, reduce the soils nutrient availability and compromise the sustainability of these systems (Joffre *et al.* 1999; Peco *et al.* 2006).

Among the proposed management options for the tree regeneration of dehesas, a spatially and temporally limited set-aside of grazing and cultivation by means of rotating fences is the one that has more often been preferred in both management and scientific literature (Gibbons *et al.* 2008; Montoya 1998; Plieninger *et al.* 2003). As an alternative, the adoption of non-continuous grazing management schemes such as transhumance has been recently proposed as a measure to ensure the regeneration of scattered trees (Dorrough 2005; Fischer *et al.* 2010). In Mediterranean systems there is a very marked drought during summer, which results in reduction in the availability and quality of herbaceous vegetation. This is associated with a great increase in both the amount of supplementary feed needed by livestock during the summer (Gutman *et al.* 2000) and in the intensity of browsing in this season compared to spring, when there is a much higher availability of fodder (de Miguel *et al.* 1997). This fact has led to the proposal of the recovery of transhumance practices as a measure to solve the problem of the lack of regeneration of the tree layer in dehesas (Garzón-Heydt 2004). Nevertheless, to date no study has tested the effects of seasonal grazing regimes on tree regeneration in dehesas.

In the present paper we analyze the effects of seasonal grazing associated with transhumant pastoralism on the regeneration of holm oaks, by comparing three specific aspects of tree regeneration and condition (browsing status, regeneration and size structure) in different dehesas with contrasting management regimes (transhumant seasonal grazing vs. permanent grazing). We hypothesize that the conservation and regeneration status of holm oak saplings and trees will be better in estates managed under transhumance regimes than in estates with permanent grazing regimes.

7.2 MATERIAL AND METHODS

Study area and sampling design

The study area is located in the municipalities of La Carolina, Vilches and Santa Elena (38°20' N, 3°30' W; 400-600 m.a.s.l.) in South-Central Spain (Fig. 7.1). The climate is typical Mediterranean, with very dry summers and annual rainfalls concentrated in spring and autumn. Annual rainfall is ca. 600 mm, and mean annual temperature ca. 17 °C. These dehesas are located in one of the wintering areas for transhumant livestock of the Conquense Drove Road, one of the major drove roads still in use on foot transhumance by sheep and cattle in Spain.

We selected 18 estates of two different types according to grazing seasonality: nine of them were grazed throughout the year (*Permanent*), while the other nine were managed by transhumant livestock raisers and grazed only for six months each year (between December and May; *Transhumant*). In each estate we randomly selected between 2 and 6 plots, always facing North, the most common orientation in the study area, in order to reduce as much as possible the ecological variability among observations. Minimal distance between plots belonging to the same estate was set to 100 m.

The estates were selected in order to cover a large range of tree densities and diverse grazing alternatives: among the estates studied within each grazing regime, some were grazed by sheep, some by cattle and some by both. We calculated for each estate the grazing pressure expressed in Livestock Units (LU) * day * ha^{-1} (1 cattle=1 LU, 1 sheep=0.15 LU), in order to have a comparable measure between estates. Grazing pressure can also vary within farms according to different factors such as topography, livestock preferences or the location of grazing management infrastructures (Carmona *et al.* unpublished). To have an estimation of the local grazing pressure, we performed livestock excrements counts in four 10x1 m transects (starting in the center of the plot towards the N, S, E and W directions).

In each plot we measured three groups of variables (browsing indicators, regeneration indicators and woodland structure indicators) to characterize the condition of the holm oaks. The browsing indicators included two variables: the intricacy index and the percentage of eaten shoots. These variables were measured in the four holm oaks nearest to the center of the plot that presented branches accessible to livestock at a height less than 1 m. In each of these oaks we randomly selected a branch

with no visible damage and with a basal diameter of 0.5 to 1 cm and a length greater than 10 cm. The intricacy index was calculated as the ratio between the number of times that the selected branch ramifies and its total length, and it is expected to increase with the intensity of browsing (Orueta *et al.* 1998). In the same oaks, we determined the percentage of eaten shoots. For this purpose, we randomly selected 10 branches of the outer part of the tree to a height of less than 1 m and counted how many of them were eaten (absent terminal bud).

We selected four 3x3 m subplots in each plot (distant 7 m from the center of the plot in the N, S, E and W directions) in which we measured the regeneration indicators. In each subplot we counted the number of isolated juvenile holm oaks (<1 cm DBH or less than 1.3 m height) and recorded their base diameter and height. In the case of groups of juveniles (very aggregated and with a similar height and leaf morphology), we assigned for the whole group the height and diameter of the highest juvenile in the group (Plieninger *et al.* 2004). We considered two

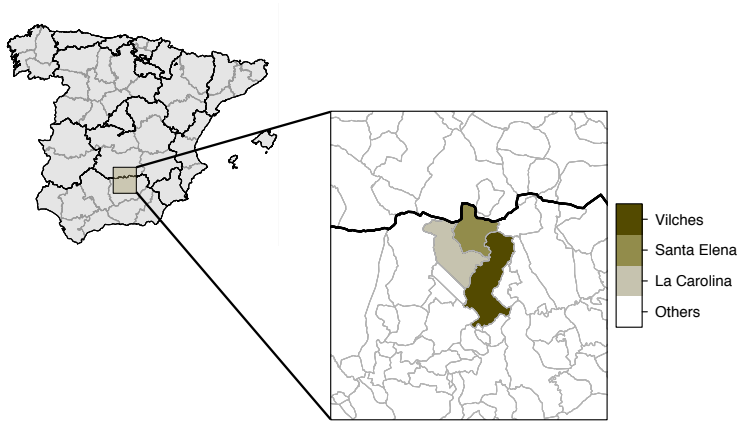

Figure 7.1. Overview of Spain and the location of the three municipalities in which the study area was situated, in northern Jaén province.

groups as different when their external borders were more than 25 cm apart (Plieninger *et al.* 2004). To estimate the cover of each juvenile or group of juveniles, we measured the longest length of the crown and the length of the perpendicular axis. For each subplot we calculated the average base diameter and height of juveniles and the proportion of the surface covered by them.

Since livestock (especially cattle) can break and consequently kill holm oaks of up to 7-9 cm (Pérez-Fernández and Gómez-Gutiérrez 1995), the woodland structure indicators were chosen in order to detect the effects of grazing on the density of three different size classes: young saplings (DBH between 1 and 5 cm), old saplings (DBH between 5 and 10 cm) and adult holm oaks (DBH > 10 cm). We first measured the distance from the center of the plot to the closest holm oak of each of the three size classes. After that, from each of those trees we measured the distance to its restricted second neighbor and applied a T-square sampling to determine the density (trees*ha^{-1}) of each size class (Besag and Gleaves 1973). We calculated a plot-averaged value for all the indicators that were measured more than once in each plot and used that average in the subsequent analyses.

Statistical analysis

We analyzed the relationships between grazing variables or factors and each of the browsing, regeneration and size structure indicators with mixed linear models. Grazing regime (GR; categorical with 2 levels: Permanent and Transhumant grazing), number of excrements (E; continuous), grazing pressure at the farm level (GP; continuous) and the type of grazer (TG; categorical with 3 levels: Sheep, Cattle and Both), were included in the models as fixed effects and farm was included as a random factor in order to account for variability among the farms due to other non-measured variables. The relationship between the grazing variables and factors was previously analyzed with t-test and chi-square in order to discard multicollinearity in the models. These tests revealed that the only variables that were significantly correlated were grazing regime and grazing pressure (t_{16}=5.60, $p<0.001$), with higher values of grazing pressure in permanently grazed than in transhumant estates (185.98 $\pm$ 14.99 and 94.20 $\pm$ 7.58 LU*day*ha^{-1}, respectively).

The response variables used (Intricacy index, percentage of eaten shoots,% of surface covered by young oaks, diameter and height of

young oaks, and density of young saplings, old saplings and mature oaks) were log-transformed in order to attain normality and homoscedasticity of residuals. Since GR and GP were correlated, we never used them simultaneously in the same model; thus, for each response variable we fitted two sets of linear mixed models. One set included GR, E, TG and all the first order interactions as fixed effects and the other set included GP, E, TG and all the first order interactions as fixed effects. These saturated models were simplified via backwards stepwise selection in order to attain two final candidate models. Among these final models, we selected the one with the lowest AIC value (Burnham and Anderson 2002) as the one that better explained the observed data. All the analyses were performed using the program R version 2.14.1 (R Development Core Team 2011).

7.3 RESULTS

Browsing indicators

Differences between estates accounted for more than 40% of the variability in browsing indicators. GR was found to be a much better predictor of these variables than GP, as indicated by the great differences in the AIC values between the models including each predictor (Table 7.1). The selected model for the intricacy index of oak saplings contained only grazing regime as a statistically significant predictor, accounting for a 15.11% of the total variability and revealed that this index was much higher in plots under permanent grazing regimes than in those used by transhumant livestock (Fig. 7.2A). As expected, the direction and magnitude of the effect of grazing regime on the percentage of eaten shoots were similar than the effect on the Intricacy index. Nevertheless, the selected model for the percentage of eaten shoots revealed a positive correlation between this variable and the amount of excrements in the plot, indicating the significant effect of within-estate variability in grazing pressure.

Stand regeneration indicators

Again, grazing regime, instead of grazing pressure, was generally included in the most parsimonious models for these variables (Table 7.1). The estate factor accounted for a 40% of the variability in the proportion of surface covered by oak juveniles, but it was less important for the diameter and height of the juveniles (Fig. 7.2B). The selected model for the

proportion of the surface covered by oaks in the regeneration stage included only grazing regime as a predictor. As expected, this proportion of surface was higher in transhumant than in permanent grazing plots. None of the studied predictors showed any effect on the diameter of the juveniles, but we observed an important effect of grazing regime on the height of these juveniles (Fig. 7.2B).

Size structure indicators

Inter-estate variability was not important for explaining the density of young saplings, but in contrast it accounted for higher proportions of the variability of the density of old saplings and especially of mature oak trees (Fig. 7.2C). Once more, the AIC values indicated that the models that included GR were more parsimonious than those including GP. Despite the fact that the average values of the density of the three size classes was higher in transhumant than in permanent grazed plots, grazing regime did not present a significant effect on the density of mature trees. We found a weak effect of grazing regime on the density of young saplings, while this variable had a more important effect in the model for the density of old saplings (DBH between 5 and 10 cm; Fig. 7.2C).

7.4 DISCUSSION

The present study provides evidence for the important effect that the early cessation of grazing associated with transhumance practices has on the regeneration of the tree layer in dehesas. There is an obvious association between grazing regime and grazing pressure, caused by the increased amount of time that animals spend in the permanently grazed estates rather than by differences in the number of animals per surface unit. Nevertheless, the most parsimonious models to predict the different indicators of the tree layer status generally included grazing regime rather than just grazing pressure. This result indicates that, not only the number of animals that graze in an estate, but also the timing of grazing determines the condition and density of the holm oaks.

Effects of grazing regime on tree regeneration

In Mediterranean dehesas, herbaceous vegetation gets completely consumed or dried during the summer. This reduction in the availability

and quality of the herbaceous vegetation (Papachristou 2005) increases the use of browse as a food resource for ungulates during summer, with the subsequent effects on saplings and juveniles (Bugalho and Milne 2003; Zamora *et al.* 2001). As shown by the browsing indicators, juvenile oaks in plots under transhumant practices clearly suffered much smaller levels of herbivory than those situated in permanently grazed plots. This reduction in browsing by herbivores was certainly caused by the earlier cessation of grazing in transhumant estates in which herds are moved to summer pasturelands as soon as primary productivity, and hence pasture availability, declines.

The differences in the rate of consumption of woody vegetation by livestock between estates with different grazing regimes resulted in sig-

Table 7.1. Predictors included in the most parsimonious linear mixed-effects models for the set of models that included grazing regime (GR) and the set of models that included grazing pressure (GP). The other predictors were the proportion of excrements (E) and the type of grazer (TG). For each model, we show its AIC value as well as the proportion of the total variance explained by the fixed effects (%VE). In bold, the lowest AIC value for each response variable is shown.

Response Variable Set of models	Most parsimonious model	AIC	%VE
Intricacy			
Grazing regime	∼ GR + E + TG + S:E	-4.48	17.54
Grazing pressure	∼ GP + E + GP:E	3.22	8.59
Eaten shoots			
Grazing regime	∼ GR + E	172.24	19.32
Grazing pressure	∼ GP + E + GP:E	180.66	12.41
Regeneration surface			
Grazing regime	∼ GR	175.06	7.86
Grazing pressure	∼ GP	177.00	5.73
Regeneration diameter			
Grazing regime	∼	67.67	—
Grazing pressure	∼	67.67	—
Regeneration height			
Grazing regime	∼ GR	85.48	13.99
Grazing pressure	∼ GP	90.36	5.86
Density young saplings			
Grazing regime	∼ GR	240.54	8.25
Grazing pressure	∼ GP + TG	244.86	7.65
Density old saplings			
Grazing regime	∼ GR	218.23	8.64
Grazing pressure	∼ E + TG	220.52	7.59
Density mature trees			
Grazing regime	∼	170.56	—
Grazing pressure	∼	170.56	—

nificant differences between their populations of holm oak juveniles. First, permanent grazing reduced the net growth rate of oak juveniles,

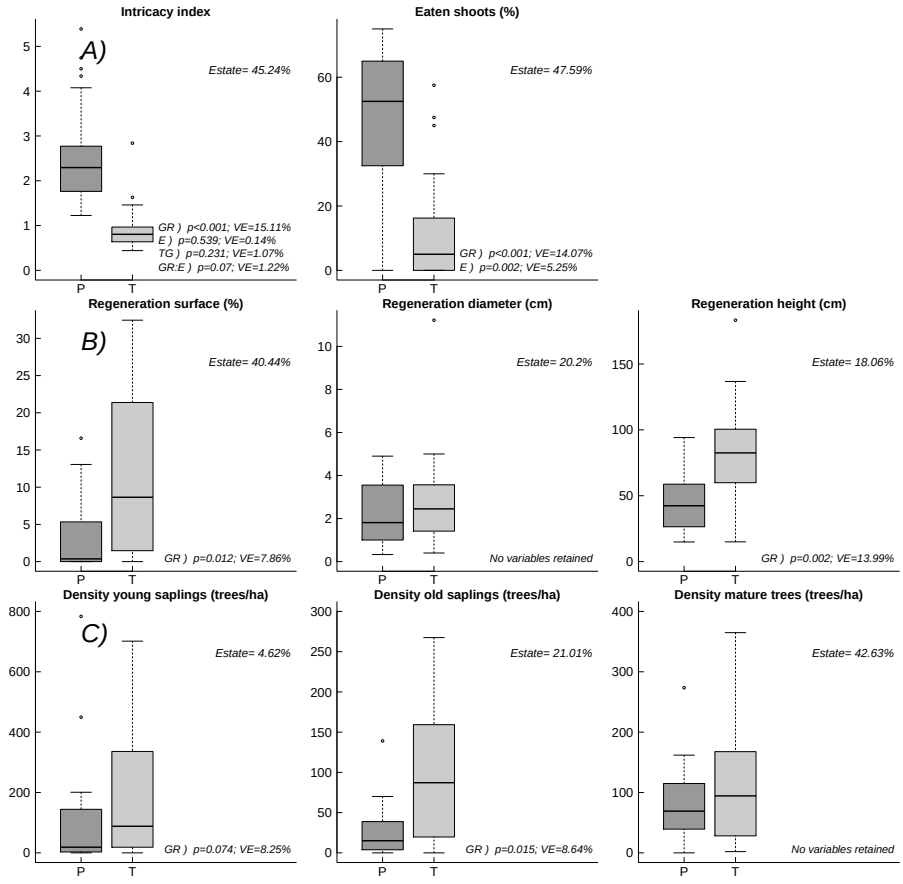

Figure 7.2. Boxplots showing the effect of grazing regime (P: Permanent grazing; T: Transhumant grazing) on the different indicators of holm oak browsing (A), regeneration (B) and size structure (C). For each predictor we show the p-values and the proportion of variance explained (VE) by each of the independent variables retained in the most parsimonious model (see text; GR: Grazing regime; E: Proportion of excrements: TG: Type of grazer). The proportion of the total variance due to differences among different estates is shown in the upper-right side of each plot.

as indicated by the smaller height of these juveniles compared to those in transhumant plots. Slower growth in juveniles suffering high levels of herbivory can have important consequences at the stand level because it can greatly increase the age required to reach the height threshold that allows the young trees to avoid being browsed (Pérez-Fernández and Gómez-Gutiérrez 1995; Zamora *et al.* 2001). Second, permanent grazing also reduced the proportion of the total surface covered by juveniles. We did not find significant differences between estates with different grazing regimes in their number of groups of juveniles, which indicates that the reduced cover in permanent grazing plots is more likely to be related with a significant reduction in the photosynthetic tissue of the juveniles, rather than with an increase in their mortality rate. These observations suggest that the development of oak juveniles is seriously reduced under continuous grazing conditions, probably because of a great reduction in their leaf area (Putman 1996).

It has to be noted that the higher cover and better condition of juveniles in transhumant estates does not necessarily secure long-term regeneration in those estates because grazers can significantly damage or even remove saplings before they reach maturity, as found in previous studies (Pérez-Fernández and Gómez-Gutiérrez 1995; Tiver and Andrew 1997). In relation with this, despite we only found a weak effect of grazing regime on the density of young saplings, transhumant plots presented on average a much higher density of old saplings (DBH between 5 and 10 cm) than permanently grazed plots. Important reductions in the density of saplings have previously been observed in grazed areas compared to abandoned ones (Dufour-Dror 2007). Our findings suggest that the likelihood of young holm oak individuals to reach the next class size is significantly reduced in estates in which grazers spend the summer and confirm that oaks of more than 5 cm DBH are sensible to livestock action. Thus, our results suggest that not only the seedling stage (Plieninger *et al.* 2004), but also the transition from the sapling to the adult phase can be considered as a critical stage determining the lack of success of holm oak recruitment in dehesas.

The great importance of the difference among estates suggests that environmental attributes such as rock cover, slope or shrub cover, that we have not included in our analyses, are important determinants of the regeneration stage of holm oaks (Plieninger *et al.* 2004). At the same time, among the grazing variables, only grazing regime presented a consistent effect on the studied holm oak status indicators. In general, grazing

regime was a much better predictor than grazing pressure at the estate level (Table 7.1). Similarly, grazing pressure at the plot level (indicated by the number of excrements), as well as the type of grazer, presented non-significant effects, in accordance with the lack of effects of grazing impact indicators on holm oak regeneration indicators observed in other studies in dehesas (Plieninger *et al.* 2003 2004; Plieninger 2007). To explain these observations, Plieninger (2007) proposed a model assuming that current stocking rates in dehesas are above the threshold for safe regeneration of holm oaks. We propose the timing of grazing and whether it is or not coupled with seasonal changes in the availability of herbaceous vegetation as a factor that should be taken into account, along with stocking rates, in order to study the failure of the tree layer regeneration in dehesas. Our results suggest that the lack of regeneration of the tree layer in dehesas can be caused not only by the increases in stocking rates experienced by these systems, but also by the recent abandonment of traditional grazing practices like transhumance, and probably by a combination of both processes. Complementary to this observation, previous studies in Mediterranean grasslands have described that seasonal grazing allows for higher stocking rates than continuous grazing with supplementary feed (Gutman *et al.* 1999). However, further research on the conditions that would ensure tree regeneration in dehesas is needed in order to determine realistic thresholds of grazing density both for continuous and non-continuous grazing regimes.

Implication for the conservation of Mediterranean dehesas

Scattered trees are a keystone feature of agricultural landscapes, which makes the problem of its lack of regeneration an issue that has to be faced urgently (Fischer *et al.* 2009; Manning *et al.* 2006). Our study shows that grazing cessation during summer and autumn can be applied to improve the regeneration of scattered trees, while substantially reducing the high opportunity costs of permanent livestock exclusion (Dufour-Dror 2007; Fischer *et al.* 2009 2010; Gutman *et al.* 1999). We propose two different management options in order to attain this objective:

1. The recovery of seasonal grazing regimes based on transhumant pastoralism.

2. The adaption of the temporally limited set-aside of grazing with rotating fences proposed in previous works, limiting this grazing

cessation to summer and autumn during a time long enough for oak saplings to reach a DBH of 10 cm.

We clearly advocate for the first option. Besides the improvement in the regeneration of the tree layer of dehesas, there is a great number of ecosystem services linked to transhumance practices. These include regulating services such as fire prevention, soil fertility, seed dispersal or biodiversity conservation as well as provisioning and cultural services, such as high quality food supply, cultural identity and traditional ecological knowledge (Bunce *et al.* 2004; Oteros-Rozas *et al.* 2012).

Multifunctional systems in the Mediterranean are considered an example of resilient social-ecological systems (Blondel 2006). More precisely dehesas and their typical scattered tree structures have been acknowledged for their contribution to ecological resilience (Manning *et al.* 2006). Transhumance is a millenary practice that has proved to combine the provision of a wide range of ecosystem services (Oteros-Rozas *et al.* 2012) with the conservation of dehesa cultural landscapes, by allowing a sustainable regeneration of the tree structure. How to realise new functions of ecosystems and still guarantee the sustainability of qualities of the past is a major concern in nearly all the old cultural landscapes in Europe (Vos and Meekes 1999). In this context, the recovery of customary management practices like transhumance should be encouraged through the development of adequate policies, in order to successfully face the challenges associated with current global environmental change.

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Chapter 8

General discussion

Changes in land-use are one of the most important drivers of the process of global environmental change that threatens the provision of services for humans (Vitousek *et al.* 1997; Chapin *et al.* 2000; MEA 2005). Among land-uses, grazing systems, probably the most extended and the highest resource demanding at a global scale (Foley *et al.* 2011), have experienced important changes in the last decades, changes that are expected to go on in the future because of the global increasing needs (Tilman *et al.* 2002; Bouwman *et al.* 2005; Robinson *et al.* 2011). The dual process of grazing intensification and abandonment that extends across European grasslands – and more specifically across Mediterranean ones – is compromising the diversity and the ecosystem services provided by these systems (Stoate *et al.* 2009; Sluiter and Jong 2006). Grazing is intimately linked to Mediterranean ecosystems, which have been shaped by domestic herbivory for several thousand years (Papanastis 1998; Blondel and Aronson 1999; Di Pasquale *et al.* 2005). Therefore, grazing is acknowledged as an essential factor for their conservation (Perevolotsky and Seligman 1998), and the consequences of grazing intensification and abandonment have been identified as an issue that deserves further research (Peco *et al.* 2005,2006; de Bello *et al.* 2005,2006; Golodets *et al.* 2009b; Tárrega *et al.* 2009).

Studying the consequences of land-use changes in grazing systems, require the examination of different aspects, because grazing affects vegetation through several mechanisms, whose effects are manifested at different levels and spatial scales, from individual plants to plant communities and landscape scale. This thesis has added some light on some of those aspects, following an order of decreasing detail. Thus, the first

chapter focused on the effects of grazing on individual species with different grazing responses. The next three chapters dealt with the effects of grazing intensification and abandonment on grassland communities. Finally, the last two chapters considered grazing at a landscape scale. In the following section, the main findings of this thesis are summarised and discussed and some lines for future research are proposed.

The effects of grazing are manifested in the different specific compositions between grazed and ungrazed areas (Noy-Meir et al., 1989; Verdú at al., 2000; Peco et al., 2005,2006b; Shitzer et al., 2008; Golodets *et al.* 2009b,2011; Bugalho et al., 2011). Whereas some species increase, other species consistently disappear (or experience a reduction in their abundance) in grazed areas. Livestock grazing affects plants through several different mechanisms, but it is not clear which of them are behind the different responses of plant species to grazing. Indeed, tolerance to herbivory has a prominent role, as confirmed by previous studies that compared the response to defoliation of increaser and decreaser species (del-Val and Crawley 2004,2005; Leoni 2009), as well as by the increase in the average SLA values of plant communities under grazing (Cingolani *et al.* 2005b; *CHAPTERS 3* and *4* of this thesis). Avoidance is also known to be one of the causes of these different responses, as indicated by the fact that grazing selects for short species and for those with rosette or prostrate growth forms (Díaz *et al.* 2007a; *CHAPTERS 3* and *4* of this thesis).

However, besides the direct effects on the individuals, grazing can also influence the species composition of a given area through indirect effects, i.e. changing the environmental conditions in which plants live. Dobarro *et al.* (2010) presented the first work comparing the differences in germination response between increaser and decreaser species under light quality and quantity conditions similar to those experienced in grazed and ungrazed areas. They found a decrease in the germination rate of increaser species under light conditions similar to those observed in ungrazed areas, and attributed this response to the ability of increasers to avoid germination under highly competitive conditions (Dobarro *et al.* 2010). The results presented in *CHAPTER 2* of this thesis indicate that dung leachates are likely to reduce the decreaser species' ability to colonize grazed areas, as well as the capacity of their seedlings to survive under water-stressed conditions (Espigares and Peco, 1995; Hamilton et al., 1999). This study is the first comparison between grazing increaser and decreaser species using confamilial pairs of species with different

grazing responses, thus reducing the likelihood of phylogenetic biases in the results (Blaney and Kotanen 2001; Morrison *et al.* 2011). A significant result from *CHAPTER 2* is the observed spatial distribution of dung pats and pellets, which indicates the high likelihood of a given site to be affected by cattle dung (Bakker and Olff 2003). This observation, along with the long time that dung take to decompose in the field (Dai 2000), suggests that leachates can have a significant effect on the compositional patterns in grasslands. Moreover, regardless on the likelihood of dung leachates affecting an individual plant, the measurements presented in *CHAPTER 3*, revealed that grazing increases soil fertility. Altogether, these results point in the same direction of those reported by Dobarro *et al.* (2010), indicating that changes in environmental conditions caused by livestock actions can differently affect different species, and consequently be one of the reasons for the different grazing responses of plant species.

However, to ascertain the real significance of the influence of grazers in the germination niche of grassland species and whether increaser and decreaser species are affected differently, these studies should be replicated in field conditions. Regarding the effect of dung leachates, field studies should focus not only on testing the differences in germination between increaser and decreaser species under the influence on dung, but also on whether these differences are likely to be significant for grassland communities. In order to achieve this knowledge, it is necessary to characterise the spatial and temporal variability of the dung leachates' effects. Therefore, future studies in field conditions should determine the reach of the influence of dung leachates coming from dung pats and whether these effects are dependent on the time since deposition. The potential implications that the reduction in root length experienced by seedlings of decreaser species could have on the capacity of these species to survive in water stressed conditions also deserves further research.

Additionally, the shape of the germination responses to leachate concentration suggests that the effects on germination did not depend on the leachate concentration, but only on its presence or absence, which in turn suggests a greater pathogen activity in the leachates environment, affecting the two kinds of species in different ways. Among these pathogens, fungi are very abundant and known to disturb seeds in many different ways (Crist and Friese 1993; Leishman et al., 2001) and can benefit from the increased nutrient availability provided by dung leachates (Johnson et al., 2008; Ruprecht et al., 2008). Seed sensitivity to fungal

attacks differs between different species and environmental conditions (Leishman *et al.* 2000a; Blaney and Kotanen 2001; Schafer and Kotanen 2004). Therefore, the presence of fungi in dung leachates or their effect on the fungi already present in the soil or in the seeds could be the cause behind the different responses between increaser and decreaser species, but new studies should be performed in order to test this hypothesis.

Despite these advances, the influence that the different livestock actions have on the composition of plant communities is still far from being understood. It has to be noted that the different grazing actions can interact among them and with other environmental variables. For instance, the germination process is influenced by the interaction between nitrogen availability and light (Vandelook *et al.* 2008), an observation that links the studies of Dobarro *et al.* (2010) and *CHAPTER 2* of this thesis. Likewise, the tolerance to grazing of some plant species is known to depend on resource availability (e.g. Staalduinen *et al.* 2010). These effects on individual plants scale-up, leading to the very context-dependent effects of grazing on plant communities (Milchunas *et al.* 1998; de Bello *et al.* 2005; Bakker *et al.* 2006). The results presented in *CHAPTERS 3, 4* and *5*, which focused on grassland communities, confirmed that grazing effects are modulated by environmental conditions.

First, in *CHAPTER 3*, the changes caused by grazing abandonment in species richness and functional trait diversity, along with its impact on floristic composition, trait values at the community level, soil fertility and bare soil cover were studied. The results revealed changes in the community average value of all analysed plant functional traits and a loss of functional diversity after abandonment in two of them, which was not associated with a change in species richness, indicating a loss of functional groups and a potential effect on ecosystem functioning (Díaz and Cabido 2001; Naeem and Wright 2003; Peco *et al.* 2006; Mayfield *et al.* 2010). Abandonment was accompanied by the disappearance of short-sized species, prostrate or creeper growth forms and early flowering species, confirming the observation of previous studies, and remarking the role of grazing as a filter that selects for plants with avoidance mechanisms (Noy-Meir *et al.* 1989; Fernández-Alés *et al.* 1993; Hadar *et al.* 1999; Lavorel *et al.* 1999; Sternberg *et al.* 2000; Peco *et al.* 2005; Castro *et al.* 2010). Besides, abandonment favoured large-seeded species and low SLA values, the latter suggesting changes towards a more conservative resource acquisition strategy in abandoned grasslands (Westoby 1998; Westoby *et al.* 2002). The changes in the functional traits of the

communities that have experienced grazing abandonment point to decreases in the nutrient recycling rates and litter decomposability, which could be behind the detected decline in soil fertility after abandonment.

However, *CHAPTER 3* only compared grazing abandoned areas with areas with moderate grazing intensity and, like previous studies in Mediterranean environments (Peco *et al.* 2005, 2006), ignored the most intensively grazed areas. *CHAPTERS 4* and *5* tried to fill this gap in knowledge by including the highest end of the grazing gradient. The inclusion of this level revealed that high grazing shifts the average trait values, confirming that high grazing pressures favour grazing avoidance (grazing favours small species; *CHAPTER 4*) and rapid resource acquisition strategies (SLA values increased with grazing; *CHAPTER 4*). It is important to note that these differences would have not been found without the incorporation of the most intensively grazed level, a result that shows the importance of the inclusion of the whole range of grazing pressures in the studies analysing the response of plant communities to grazing.

The dataset collected for these chapters included communities in two habitats differing in their productivities, that were sampled in two years with contrasting rainfall levels. This allowed for the study of the changes in assembly processes associated to interannual changes in water availability along spatial gradients of resource availability and disturbance, presented in *CHAPTER 4*. The use of appropriate null models (Gotelli and McCabe 2002) allowed the estimation of pure measurements of functional richness and functional divergence, the two components of functional diversity with the highest power to detect changes in community assembly processes across environmental gradients (Mason *et al.* in press). The observed effects on vegetative traits (height and SLA) were generally consistent with the expected shift from size-symmetric competition for belowground resources in dry years to size-asymmetric competition for light in wet years (Casper and Jackson 1997; Schiwinning and Weiner 1998; Berntson and Wayne 2000; Mason *et al.* 2012,in press). These results support the role of pulses in water availability as a major determinant of plant diversity in semi-arid communities, because they reduce the differences in average fitness between co-occurring species (Chesson *et al.* 2004). Besides, the patterns in functional richness for seed mass contrasted with those of the vegetative traits, revealing the simultaneous existence of trait convergence and divergence for different niche axes (Grime 2006; Spasojevic and Suding 2012). Altogether, the results

of this chapter highlighted the importance of the use of a combination of complementary measures of functional diversity based on individual traits rather than on multiple combinations of traits for the study of assembly processes.

CHAPTER 5 focused on the study of taxonomical diversity and its relationship with functional diversity, in an attempt to achieve a better understanding of the effects that human activities have on grassland ecosystems (de Bello *et al.* 2005,2006; Díaz *et al.* 2007c; Sasaki *et al.* 2009b). The combined effects of grazing intensity and water availability on plant taxonomic and functional diversity and its relationship across different spatial scales were studied. The results revealed that the main factor determining both species and functional diversities was the type of habitat (highly related to water availability) and, more importantly, that water availability modulated the effect of grazing within and across communities (Cingolani *et al.* 2005a; de Bello *et al.* 2006). During the dry year, dry habitats' plant communities experienced drastic reductions in their values of taxonomic diversity, but not in functional diversity, which indicated that functional and taxonomical diversities are decoupled in dry habitats. The results of the spatial partition of diversity confirmed the interpretation of grazing and aridity as convergent selective forces (Milchunas *et al.* 1998; Quiroga *et al.* 2010) and the high levels of functional redundancy in dry habitats. Nevertheless, under severe restrictions in water availability, high grazing pressures could reduce functional diversity, revealing a convergence in traits under the combined effect of grazing and drought conditions, a feature that had also been observed in *CHAPTER 4* for individual traits. The study presented in *CHAPTER 5* improves the still limited knowledge about the relationship among functional and species diversities and about diversity partitioning across spatial scales (Micheli and Halpern 2005; de Bello *et al.* 2009; Golodets *et al.* 2011; Biswas and Mallick 2011) and is the first considering the two approaches simultaneously. Bearing in mind the expected reduction in water availability in Mediterranean environments, the adoption of flexible grazing management schemes that take into account simultaneously species and functional diversities and adapt the level of grazing pressure depending on water availability was recommended.

Altogether, the results presented in *CHAPTERS 3, 4* and *5* revealed that grazing and productivity are important determinants of the assembly processes of plant communities. Nevertheless, our understanding of the processes determining the assembly of biological communities is

still very limited. For example, average trait values are regularly used to characterize each species, assuming that interspecific variability is much more important than intraspecific variability. However, this assumption is not necessarily truth, and a growing body of evidence indicates that intraspecific and individual variability can have significant ecological effects (Mason *et al.* 2011; Bolnick *et al.* 2011; Violle *et al.* 2012). Consequently, new analytic tools are being developed in order to satisfy the need of including intraspecific diversity into the analyses of functional diversity indices (Violle *et al.* 2012; de Bello *et al.* in press). The results provided by these studies suggest that, in many occasions, taking intraspecific variability into account can improve our power to detect assembly processes, and also provides measurements of functional diversity that are less dependent on the local species pool (de Bello *et al.* in press). The use of these new techniques in the analysis of plant communities' responses to changes in land-use and productivity would be a major advance in our understanding of the effects that these features have on ecosystem processes.

The importance of interannual rainfall fluctuations in the determination of the assembly processes of Mediterranean grasslands communities is one of the most important results of this thesis. However, the analysed dataset included only two years with contrasting amounts of rainfall. The analysis of longer time series would be a major step in our understanding of the processes that determine diversity in semi-arid grassland communities, and one that is particularly important in the light of the predicted reduction in rainfall in these environments (Chapin *et al.* 2000; Christensen *et al.* 2007).

As stated before, there are many open questions about the effects that the different actions of grazers have on plant communities. Some studies have tried to decompose livestock actions by experimentally simulating its effects on field conditions (Kohler *et al.* 2004, 2006; Dobarro 2009; Gillet *et al.* 2010). However, these approaches have not explicitly studied the effects of livestock actions in functional diversity, or have only taken into account the average trait values (Kohler *et al.* 2006). A combination of the two approaches – simulated grazing effects and rigorous analysis of assembly processes – would significantly improve our understanding of the significance and effect that the different livestock actions have on plant diversity.

Finally, the last two chapters presented in this thesis dealt with the consequences of grazing at a landscape scale. In *CHAPTER 6*, a

methodology to support rangeland management and assess grazing effects (Thompson *et al.* 2009; Kleinebecker *et al.* 2011) on environmentally heterogeneous areas at a landscape scale was presented and applied in a Mediterranean dehesa. This is the first study dealing with the response of herbaceous vegetation to grazing at a landscape level in Mediterranean environments. The results from the example revealed that the recovery capacity maximized on intermediate grazing pressures for humid habitats, suggesting the existence of an optimal level of grazing in these zones, while in dry habitats vegetation response decreased consistently along with grazing pressure. These results concurred with those presented in *CHAPTERS 4* and *5*, and provided further support for the importance of the complex interactions between grazing pressure, soil fertility and water availability as determinants of ecosystem processes in grasslands (Shariff *et al.* 1994; Biondini *et al.* 1998; Bardgett and Wardle 2003; Augustine and McNaughton 2006; Blanco *et al.* 2008). The presented methodology provided a valuable insight into how different variables and its interactions affect the recovery capacity of vegetation and can be used as a tool in the management of highly heterogeneous rangelands.

Nevertheless, there are several aspects that could be improved in the methodology presented in *CHAPTER 6*. Specifically, the calculation of the cost surfaces used to infer grazing pressure could be improved by taking into account some new aspects. First, the modelling of the spatial distribution of livestock across the studied landscapes would be improved by taking into account the relative importance on the points of livestock concentration (e.g. Paudel and Andersen 2010); this step would also allow the simultaneous study of different farms at a local or regional scale. Second, a rigorous quantification and inclusion of the feeding preferences of livestock in the cost surface calculation would certainly improve the reliability of the models (e.g. Röder *et al.* 2007). Finally, more realistic values for the parameters used in the friction surfaces could be achieved by studying the behaviour of livestock under differing topography and accessibility conditions. The study of data from livestock wearing GPS collars in actual landscapes would help to achieve this goal (e.g. Ungar *et al.* 2005). Besides, the estimation of the effect of grazing on vegetation dynamics would greatly benefit from a more continuous estimate of vegetation cover, rather than using only two observations, in the dry and wet seasons. The reduction in costs and

the increased availability of high spatial resolution images are likely to make this improvement possible in the next years.

A remarkable finding from *CHAPTER 6* is the small reduction in vegetation cover during the dry season in the proximity of trees. This result confirmed the importance of trees as a fundamental feature in dehesa systems (Marañón *et al.* 2009; Manning *et al.* 2009). However, several authors have described an extensive regeneration failure of oaks, which compromises the long-term stability of dehesas (Montoya 1998; Plieninger *et al.* 2003; Plieninger 2007; Moreno and Pulido 2009), a problem that has to be faced urgently. In order to cope with this problem, different management alternatives, such as the temporal or permanent abandonment of grazing and the reduction in the stocking rates, have been suggested (e.g. Montoya 1998; Plieninger *et al.* 2003; Gibbons *et al.* 2008). However, and despite temporal cessation of grazing has been recently suggested as an option to reverse the global decline in scattered trees (Dorrough 2005; Fischer *et al.* 2010), the effect of the recovery of non-continuous grazing practices such as transhumance has not been tested to date. In *CHAPTER 7*, the effects of seasonal grazing associated with transhumant pastoralism (Ruiz and Ruiz 1986; Manzano and Casas 2010; Weber and Horst 2011) on the regeneration of holm oaks were analyzed, comparing three specific aspects of tree regeneration and condition (browsing status, regeneration and size structure) in different dehesas with contrasting management regimes (transhumant seasonal grazing vs. permanent grazing). In the models fitted to analyze the different indicators of the tree layer status, grazing regime was consistently preferred over grazing pressure, indicating that, not only the number of animals that graze in an estate, but also the timing of grazing determines the condition and density of the holm oaks. Oak juveniles were remarkably less browsed and occupied a much higher surface in transhumant than in permanently grazed estates. As a consequence, the density of saplings was higher in transhumant estates. The results suggested that the lack of oak regeneration in dehesas could be caused not only by the increases in stocking rates, but also by the recent abandonment of traditional grazing practices like transhumance. These results have clear implications for the management of dehesas, especially because the lack of regeneration of their trees is an issue that has to be faced urgently. The recovery of seasonal grazing regimes based on transhumant pastoralism was proposed as a measure to improve the conservation status of dehesas.

However, the study presented in *CHAPTER 7* describing the effects of transhumant and permanent grazing in the regeneration of holm oaks in dehesas was only the first effort to characterise the differences between the two different management options. A precise determination of the carrying capacity thresholds for permanent and transhumant grazing is the next step that should be taken in order to improve the conservation and management of dehesa systems. Besides, and although the recovery of traditional transhumant practices is proposed as the most desirable option, there are other alternatives that could be more easily implemented in the current socio-economical context. These alternatives should include the cessation of grazing during summer to promote the regeneration of trees, which could be achieved either at the whole-farm level – with the use of summertime indoor breeding or motorized transhumance equivalents – or by the designation of farm sectors in which tree regeneration is promoted by the prevention of summer grazing, until oak saplings reach a DBH threshold of 10 cm. Future studies comparing the feasibility of these alternatives would substantially help in the design and implementation of adequate policies to assure the long-term stability of dehesa systems.

Chapter 9

Conclusiones

De los trabajos desarrollados en la presente tesis doctoral se extraen las siguientes conclusiones generales:

1. Los efectos que los lixiviados de excrementos tienen en la germinación de las semillas y el desarrollo inicial de las plántulas de las especies de pastizales pueden ser uno de los factores que causan sus diferentes respuestas al pastoreo. La presencia de lixiviados de excrementos redujo los indicadores de germinación de las especies no favorecidas por el pastoreo, mientras que no afectó a las favorecidas.

2. La riqueza de especies no se vio afectada por el abandono del pastoreo; sin embargo, en las zonas abandonadas se observó una disminución de la frecuencia de especies de pequeño tamaño, con porte postrado o rastrero y floración temprana y un incremento de las especies con SLA alto y semillas grandes. La reducción en la diversidad funcional de algunos caracteres asociada al abandono del pastoreo apunta a una pérdida de grupos funcionales, con posibles efectos sobre el funcionamiento de estos ecosistemas.

3. Las fluctuaciones temporales en la disponibilidad hídrica causadas por las variaciones interanuales en las precipitaciones se revelaron como un factor determinante en el ensamblaje de las comunidades de pastizales mediterráneos. En el año húmedo los niveles de diversidad funcional de los caracteres relacionados con la adquisición de luz fueron más altos que en el año seco. Este resultado

sugiere que en años secos dominan los procesos de competencia por los recursos del suelo, que es simétrica con respecto al tamaño, mientras que en años húmedos domina la competencia por la luz, asimétrica con respecto al tamaño.

4. Los valores elevados de diversidad funcional para el peso de semilla estuvieron frecuentemente asociados con valores bajos de diversidad funcional para los caracteres vegetativos y viceversa, lo que revela la existencia simultánea de convergencia y divergencia para diferentes indicadores del nicho de las especies. Este resultado recalca la importancia de analizar caracteres individuales en lugar de índices que combinan diferentes caracteres funcionales cuando se estudia la formación de comunidades.

5. La riqueza funcional y la divergencia funcional se comportaron de manera distinta en muchas ocasiones, subrayando la importancia del uso simultáneo de diferentes índices de diversidad para el análisis de los efectos de las variables ambientales en los procesos de ensamblaje de comunidades.

6. La disponibilidad hídrica se reveló como un factor determinante de los efectos del pastoreo sobre la vegetación. En condiciones de alta disponibilidad hídrica, altas presiones de pastoreo estuvieron asociadas a elevados niveles de diversidad taxonómica. Sin embargo, los niveles altos de pastoreo hicieron disminuir la diversidad taxonómica y funcional en condiciones secas. La capacidad de recuperación de la vegetación en los hábitats húmedos se maximizó a niveles intermedios de pastoreo, lo que sugiere la existencia de un nivel óptimo de pastoreo en dichos hábitats, mientras que en los hábitats secos esta capacidad de recuperación decreció de manera constante a medida que aumentó la presión ganadera.

7. Se observaron grandes fluctuaciones interanuales en la diversidad taxonómica en los hábitats secos, lo que contrastó con una gran estabilidad en su diversidad funcional; esto indica que estos dos aspectos de la diversidad están desacoplados frecuentemente. Este resultado subraya la importancia del estudio simultáneo de ambas facetas de la diversidad.

8. El pastoreo y la aridez se confirmaron como fuerzas convergentes que seleccionan valores similares para los caracteres funcionales de las plantas.

9. El momento en el que el pastoreo tiene lugar tiene un importante efecto en el estado vegetativo y la densidad de las encinas en las dehesas. El abandono de las prácticas tradicionales como la trashumancia se perfila como uno de los factores que pueden estar detrás del problema de la falta de regeneración del arbolado en estos sistemas.

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